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CONTENTS

No. 1 (February 1957)

Original Articles: PAGE

647. Winter feeding of dairy cows. IV. The influence of four levels of concentrate feeding in addition to a basal ration of grass products on the production obtained from milking cows. W. Holmes, D. Reid, D. S. MacLusky, R. Waite and J. N. Watson 1

648. Weight changes in grazing cows. C. C. Balch and C. Line . . 11

649. The effect of design of teat-cup liners on mastitis incidence. F. H. Dodd, J. Oliver and F. K. Neave 20

650. The influence of temperature on the generation time of bacteria commonly found in milk. W. Yotis and R. Teodoro . . . 27

651. The lactose-chloride contributions to the freezing-point depression of milk. II. Examination of partial contributions over the full lactation period of two cows. E. R. Cole, J. B. Douglas and M. Mead . 33

652. Microscopic appearance of fat on the milk surface as affected by application of surface-active substances to the surface. N. King . 43

653. A calorimetric study of milk, cream and the fat in cream. L. W. Phipps 51

654. A rapid method for estimating the size distribution of fat globules in cream. R. M. Dolby 68

655. The effect of pH on the extent of splitting or clumping of fat globules caused by agitation of hot cream. R. M. Dolby 77

656. Factors influencing the vitamin content of milk fat. II. The influence of botanical composition of the pasture. N. A. Worker and W. A. McGillivray 85

657. Factors influencing the vitamin content of milk fat. III. Seasonal changes in the vitamin A and carotenoid content of the blood plasma and milk fat of cows on pasture. W. A. McGillivray . . . 95

658. Factors influencing the vitamin content of milk fat. IV. The source of milk fat vitamin A and carotene. W. A. McGillivray . . . 102

659. Factors influencing the vitamin content of milk fat. V. Changes in vitamin A and carotenoid content of the blood plasma of cows and heifers near parturition in relation to secretion of these substances in the milk fat. S. Y. Thompson and W. A. McGillivray . . . 108

660. Properties and composition of colostrum from Egyptian buffaloes and cows. A. M. El Negoumy 115

Reviews of the Progress of Dairy Science:

Section F. Milk-borne disease. J. Smith 121

No. 2 (June 1957)

ORIGINAL ARTICLES: PAGE

661. The genetic connexion between body size, milk production and efficiency in dairy cattle. I. L. MASON, ALAN ROBERTSON and B. GJELSTAD 135

662. The comparative efficiency of some methods of estimating the live weight of dairy cows. A. W. A. BURT 144

663. The relationship between milk secretion and the rate of milking by machine. P. A. CLOUGH and F. H. DODD 152

664. The effect of thyrotropin on milk yield and heart rate of the goat. E. C. OWEN and R. A. DARROCH 157

665. Mammary gland development in the calf as a possible predictor of subsequent milk production. GILLIAN M. ELLIOTT 162

666. Intramammary pressure changes in the lactating ewe. I. The nature of the pressure change under standard conditions. W. G. WHITTLESTONE 165

667. Synthesis of lactose *in vitro* by mammary gland slices from lactating rats, guinea-pigs and sheep. W. G. DUNCOMBE 171

668. The influence of cream rising upon the activity of bacteria in heat-treated milk. R. C. WRIGHT and J. TRAMER 174

669. Steam distillation of taints from cream. III. Factors affecting rate of removal of the reference substances, diacetyl and acetoin. F. H. McDOWALL 184

670. Further studies of the effect of processing on some vitamins of the B complex in milk. HELEN R. CHAPMAN, J. E. FORD, S. K. KON, S. Y. THOMPSON, S. J. ROWLAND, E. L. CROSSLEY and J. ROTHWELL 191

671. The association between the fat percentage of cows' milk and the size and number of the fat globules. J. O. L. KING 198

672. Variations in the solids-not-fat content of milk. Investigations into the nature of the solids-not-fat problems in the west Midlands. T. W. GRIFFITHS and J. FEATHERSTONE 201

673. Bacteriology of Cheddar cheese. A study of starter organisms in manufacture and maturing. D. J. DAWSON and J. T. FEAGAN . . 210

674. The lactic acid and volatile fatty acid contents of five locally made type cheeses. A. J. G. BARNETT and G. A. TAWAB 225

675. The carbon dioxide content of New Zealand Cheddar cheese. P. S. ROBERTSON 235

PAGE

676. Diffuse reflexion spectra of dairy products in the near infra-red region. J. D. S. Goulden 242

Reviews of the Progress of Dairy Science:

Section A. Physiology of dairy cattle. Hormones in reproduction and lactation. G. K. Benson and A. T. Cowie 252

No. 3 (October 1957)

Original Articles:

677. The effect of variations in nutrient intake upon the yield and composition of milk. I. Variations in the amount and type of concentrates fed in winter rations. A. W. A. Burt 283

678. The effect of variations in nutrient intake upon the yield and composition of milk. II. Factors affecting rate of eating roughage and responses to an increase in the amount of concentrates fed. A. W. A. Burt 296

679. The relationship between milking rate and milk yield. Öystein Sandvik 316

680. Some properties of the mammary myoepithelium of the sow. W. G. Whittlestone 321

681. The chemical composition and the cell count of milk. R. Waite and P. S. Blackburn 328

682. Some results on the variability of the clot-on-boiling test. Zena D. Hosking and H. Barkworth 340

683. Factors influencing the vitamin content of milk fat. VI. The effect of reduced carotene intake. W. A. McGillivray and N. A. Worker . 346

684. Factors influencing the vitamin content of milk fat. VII. The possible existence of protein-bound carotenoids and vitamin A alcohol in mammary secretion. W. A. McGillivray 352

685. Factors influencing the destruction by heat of vitamin B_{12} in milk. J. E. Ford 360

686. The fatty acids of butterfat and the volatile acids formed on oxidation. J. C. Hawke 366

687. Changes in size of fat globules in cream during Vacreator treatment. R. M. Dolby 372

688. Phage-organism relationships among strains of *Streptococcus cremoris*: the selection of strains as cheese starters. H. R. Whitehead and Elizabeth J. Bush 381

Reviews of the Progress of Dairy Science:

Section B. Bacteriology and mycology applied to dairying. E. R. Hiscox and C. A. E. Briggs 387

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CONTENTS OF THE OCTOBER ISSUE

General Meeting.

Immersion Cleaning, by Dr. C. C. THIEL.

Testing Milk Tanks, by H. S. HALL.

The Effect of Heat on the Colour of Milk, by H. BURTON.

Wrapping Materials for Dairy Products, by W. G. WEARMOUTH.

Shinfield Conference.

Experience with Aluminium Milk Cans, by J. C. BAILEY and A. W. BRACE.

Yoghurt and other Cultured Milks, Part III, by J. G. DAVIS.

Scottish Milk Testing Scheme, by C. H. CHALMERS.

Pathogenic Organisms in Dairy Products, by J. SMITH.

British Standards.

Correspondence. Obituaries.

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CONTENTS OF VOL. IX, No. 3, SEPTEMBER 1956

Annual Genetic Gain Due to Selection in a Buffalo Herd. O. P. AGARWALA.

A Study of Variations in Butterfat Content of Bulked Milk from Hariana Cows. L. D. AHUJA and A. N. GAUTAM.

Hydrogen-Ion Concentration of Milk. Part II. Effect of Some Factors on the pH of Milk. M. BHIMASENA RAO and NOSHIR N. DASTUR.

The Size of Fat Globules and the Creaming Power of Cow, Buffalo, Sheep and Goat Milk. A. H. FAHMI, I. SIRRY and A. SAFWAT.

Riboflavin Content of Buffalo Milk Curds. T. J. BOMAN and (Miss) USHA C. DALAL.

Seasonal Variations in the Reichert and Iodine Values of Milkfat. S. R. SAMPATH and C. P. ANANTAKRISHNAN.

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Vol. 16, No. 2 *Contents* *April 1957*

KLEMPERER, R. M. M. The normal distribution of the resistance of coliphage T3 to inactivation by bacterial receptors and its application to their assay.

BOULTER, D. The oxidases of *Gelasinospora tetrasperma* in relation to the endogenous respiration.

FUCHS, A. and BONDE, G. J. The nutritional requirements of *Clostridium perfringens*.

FUCHS, A. and BONDE, G. J. The availability of sulphur for *Clostridium perfringens* and an examination of hydrogen sulphide production.

FRY, B. A. Basic triphenylmethane dyes and the inhibition of glutamine synthesis by *Staphylococcus aureus*.

FINGER, I. Immunological studies of the immobilization antigens of *Paramecium aurelia*, variety 2.

KIRKHAM, D. S. The relationships between cultural characters and pathogenicity in *Venturia inaequalis* and *V. pirina*.

FAHRAEUS, G. The infection of clover root hairs by nodule bacteria studied by means of a simple glass slide technique.

BISSET, K. A. and PEASE, P. The distribution of flagella in dividing bacteria.

UDEN, N. VAN and SOUSA, LIDIA DO CARMO. Yeasts from the bovine caecum.

MEYNELL, G. G. The applicability of the hypothesis of independent action to fatal infections in mice given *Salmonella typhimurium* by mouth.

KLECZKOWSKI, A. A preliminary study of tobacco mosaic virus by the gel diffusion precipitin tests.

ROSS, K. F. A. and BILLING, E. The water and solid content of living bacterial spores and vegetative cells as indicated by refractive index measurements.

SMITH, E. E. B., MILLS, G. T. and HARPER, E. M. A comparison of the uridine pyrophosphoglycosyl metabolism of capsulated and non-capsulated pneumococci.

FINCHAM, J. R. S. and BOYLEN, J. B. *Neurospora crassa* mutants lacking argininosuccinase.

NEWMEYER, D. Arginine synthesis in *Neurospora crassa* genetic studies.

HOBSON, P. N. and MANN, S. O. Some studies on the identification of rumen bacteria with fluorescent antibodies.

SHILO, M. The enzymic conversion of the tartaric acids to oxaloacetic acid.

SHILO, M. and STANIER, R. Y. The utilization of the tartaric acids by pseudomonads.

NAUDÉ, W. DU T. and POLSON, A. Interference between active and ultraviolet-irradiated Rift Valley fever virus.

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647. WINTER FEEDING OF DAIRY COWS

IV. THE INFLUENCE OF FOUR LEVELS OF CONCENTRATE FEEDING IN ADDITION TO A BASAL RATION OF GRASS PRODUCTS ON THE PRODUCTION OBTAINED FROM MILKING COWS

BY W. HOLMES,* D. REID, D. S. MACLUSKY, R. WAITE AND J. N. WATSON

The Hannah Dairy Research Institute, Kirkhill, Ayr

In a previous experiment (1), in which dried grass and grass silage were fed *ad lib.* to milking cows, it was found that when the amount of concentrates fed was increased from 2 to 4 lb./gal. of milk an increase of only 1·2 lb. milk/cow/day was obtained. Because grass silage is more widely used than dried grass in Great Britain, another experiment was made in which dried grass and hay were fed to milking cows in limited amounts and as much grass silage provided as the cows would eat. Concentrates were fed at four different rates for each gallon of milk produced to determine the response in animal production and milk quality over a wide range of feeding levels. Since the effect of different protein contents in the concentrates used in the previous experiment had been small, it was decided to use a single concentrate mixture of medium protein content in the present experiment.

EXPERIMENTAL

Eight autumn calving Ayrshire cows from the Institute herd were used, seven of which had calved from 77 to 100 days and one 58 days before the experiment began. Four cows had completed one lactation, and the other four from three to eight lactations. Milk yields in their previous lactations had ranged from about 500 to 1000 gal.

After the cows had calved their diet was changed gradually from pasture plus concentrates, to pasture, grazed kale and concentrates. The cows were then housed in a byre for 4 weeks before the experiment began and were fed concentrates, silage, dried grass and hay. During the experimental period the cows were allowed out daily for weighing and for a brief exercise walk.

Design of the experiment

The cows were divided into two groups of four, one group containing the older, higher yielding cows and the other the lower yielding cows in their second lactation. Within each group the cows were allocated at random to one of the four sequences of a balanced Latin square design (2), with treatments A, B, C and D (detailed below). Each period lasted for 4 weeks. The first week was regarded as introductory and the other 3 weeks as experimental.

The design of the experiment was as follows:

	Periods			
Cows	1	2	3	4
1	A	B	C	D
2	B	D	A	C
3	C	A	D	B
4	D	C	B	A

The experiment lasted from 17 December 1954 to 8 April 1955.

* Now at Wye College, near Ashford, Kent.

Rations

The four concentrate feeding treatments compared were: A, no concentrates; B, 2 lb.; C, 4 lb.; and D, 6 lb. concentrates/gal. of milk. The composition of the concentrate mixture is given in Table 1. Grass silage was offered to all cows in quantities sufficient to satisfy their appetites; those on treatments A and B received three feeds per day, and those on treatments C and D, two feeds. In addition, all cows were fed a standard ration of 6 lb. baled dried grass and 6 lb. hay/day. The analyses and calculated feeding values of the roughages are shown in Table 1.

Table 1. *The composition and calculated feeding value of the feeds used*

The concentrate mixture

Parts by weight		Composition as fed	
Oats	2½	Dry matter (%)	84·2
Barley	3	*Starch equivalent	64·1
Beans	2	*Digestible crude protein (%)	13·4
Beet pulp	1¼	Crude protein (%) (by analysis)	17·6
Soya bean meal	¾		
Dairy cubes	1¼		
Grass cubes	1		
Fish meal	⅛		

The bulk feeds

	Dry matter (%)	Crude protein (% dry matter)	S.E. (% dry matter)	D.C.P. (% dry matter)
Dried grass				
Period 1	89·1	16·8	60	12·5
Period 2	87·7	17·4	60	12·5
Period 3	86·3	17·1	60	12·5
Period 4	85·2	15·9	60	12·5
Grass silage				
Period 1	17·9±0·46	10·6	50	7·5
Period 2	19·0±0·44	10·7	50	7·5
Period 3	18·2±0·84	10·4	50	7·5
Period 4	19·6±0·48	10·8	50	7·5
Hay				
Period 1	82·2	7·6	33	3·0
Period 2	82·3	7·3	33	3·0
Period 3	82·2	8·0	33	3·0
Period 4	83·4	8·1	33	3·0

* Calculated from feedingstuff tables (5).

In each period the change of treatment was spread over the entire introductory week. The initial allowance of concentrates for each cow was based on her average milk yield in the 3 weeks before the experiment began, with a slight reduction appropriate to her rate of decline in lactation. Thereafter, the equalized feeding principle suggested by Lucas (3) was adopted, and the amount of concentrate fed per cow was reduced at the end of the introductory week of each period according to the ratio of the total yield of all cows in that week to the total yield in the introductory week of the first period.

Records and sampling

The feeds were individually weighed for each cow every day throughout the experiment. On 4 days in each week the quantities of each feed refused were also weighed. In each 21-day experimental period there were, therefore, 12 days on which feed consumption was determined. These are considered to be representative of the period as a whole.

Samples were taken of each feed in each day when feed consumption was determined. The dry-matter content of three samples of the silage and of two samples each of the dried grass and hay were determined daily. The dry matter content of two samples of the concentrate mixture was determined once a week. Bulked weekly samples of each feed were analysed for crude protein. The average dry-matter and protein contents in each period are shown in Table 1.

In an attempt to reduce variations in live weight due to body fill, the cows were weighed on five consecutive days in each week. Weighing was done at 2.45 p.m., the cows having received a uniform feed of 28 lb. grass silage at 10.30 a.m.

The cows were milked twice daily and milk yields recorded to the nearest quarter pound at each milking. Milk samples for chemical analysis were taken from the milk of each cow at the end of each week from consecutive evening and morning milkings. These two samples were mixed for each cow in proportion to yield and analysed for total solids, fat, crude protein, casein and lactose, by methods already described. (4)

Calculation of feeding level

The level of feeding of S.E. and D.C.P. as a percentage of the cow's requirements according to Woodman's standards (5) was calculated for each cow in each experimental period. In making these calculations the S.E. and D.C.P. values for hay and silage shown

Table 2. *Feed dry-matter consumption*

Treatment	Average consumption (lb./cow/day)			Daily dry-matter intake as % of live weight
	Total D.M.	Concentrate D.M.	Grass D.M.	
A	23·7	—	23·7	2·24
B	25·2	4·6	20·6	2·38
C	26·7	9·2	17·5	2·51
D	28·9	13·6	15·3	2·71
Standard error of mean	±0·5	—	±0·60	±0·05
Increase in dry-matter consumption for each additional lb. of concentrates fed per gallon of milk (by regression)				
For all cows	0·86***	2·27	−1·41***	0·08***
Higher yielding group	1·05***	2·57	−1·52***	0·09***
Lower yielding group	0·67*	1·97	−1·30***	0·07***
Periods				
1	28·6	8·4	20·2	2·70
2	26·9	7·3	19·6	2·54
3	24·6	6·2	18·4	2·32
4	24·4	5·4	19·0	2·28
Standard error of mean	±0·05	—	±0·60	±0·05
Coefficient of variation, %	5·9	—	8·9	5·6

In this and the following tables: *** denotes $P < 0{\cdot}001$; ** denotes $P = 0{\cdot}01$–$0{\cdot}001$; * denotes $P = 0{\cdot}05$–$0{\cdot}01$.

in Table 2 were taken from values given by Watson (6) and by Dodsworth & Campbell (7) for feeds of similar quality. The S.E. and D.C.P. values for dried grass were estimated from the determined crude protein contents using Watson's regression equations (6). The nutritive value of the concentrate ration was determined from the values given by Woodman (5) for the individual constituents.

RESULTS

The group of older cows gave completed lactation yields of about 9000 lb., while the group of second lactation cows gave about 6590 lb. It was found that the higher and lower yielding groups of cows differed in their reaction to the treatments, the higher yielding groups being more responsive. Because such differences are of importance, a measure of the response to treatment is given in the tables for the eight cows together, and also for each group separately.

Feed consumption

Dry matter

The average quantities of total dry matter, concentrate and grass dry matter consumed per cow are given in Table 2 for each treatment and for each period. The amount of concentrates consumed increased from treatments A to D as intended, and as it increased the consumption of hay declined slightly and that of silage declined considerably. The quantity of dried grass eaten was unaffected by the treatments. The average daily consumption of total dry matter declined from period to period because the amount of concentrates fed was reduced as the milk yield declined and there was not a corresponding increase in the amount of grass dry matter consumed.

For each increase of 1 lb. in the amount of concentrates fed per gallon of milk (Table 2) consumption of concentrate dry matter increased by 2·27 lb./cow/day, but the average consumption of grass dry matter decreased by 1·41 lb./cow/day. The net increase in daily dry-matter consumption for each increase of 1 lb. in the amount of concentrates fed per gallon of milk was, therefore, only 0·86 lb./cow/day. At each change of treatment the increase or decrease in the consumption of grass dry matter was expressed as a percentage of the amount of concentrate dry matter which replaced the grass when the level of concentrate feeding rose, or which the grass replaced when the level of concentrate feeding declined. On average, changes in the amount of grass dry matter were 60% of the changes in the amount of concentrate dry matter consumed, but for upward changes in the amount of concentrates consumed this value was 73%, while for downward changes it was only 47%.

The amount of dry matter consumed per cow per day rose from the equivalent of 2·24% of the average live weight on treatment A to 2·71% on treatment D (Table 2).

Silage dry-matter consumption

Within each period analysis of variance showed that the variation in dry-matter content between silage subsamples within any one day was low, and that there were significant variations in dry-matter content from day to day, over a range of from 15 to 22%. (The coefficients of variation were, 5·4, 3·6, 7·8 and 3·7% in periods 1 to 4 respectively.)

Regressions of daily silage dry-matter intake on dry-matter content were calculated for each treatment, and the regression coefficients were each found to be highly significant (0·01 level) and to differ significantly from each other. These coefficients showed that for each increase of 1 unit in silage dry-matter percentage there were increases in daily silage dry-matter consumption per cow of 1·18, 0·77, 0·49 and 0·42 lb. on treatments A to D respectively, i.e. the change in silage dry-matter intake per unit change in dry-matter content became smaller as the amount of concentrate fed per gallon of milk increased.

A regression was calculated to determine to what extent variations in silage dry-matter intake affected the total dry-matter intake. This showed that for each increase of 1 lb. in silage dry-matter intake, there was an increase of 1 lb. in total dry-matter intake. The variations in silage dry-matter intake were not, therefore, compensated by opposite variations in the intake of other bulk feeds. There were no differences between treatments in this respect.

Starch equivalent and digestible crude protein

The average daily quantities of starch equivalent (S.E.) and digestible crude protein (D.C.P.) estimated to have been eaten on each treatment are shown in Table 3. There were average increases in intake of 1·03 lb. S.E. and 0·24 lb. D.C.P. per 1 lb. increase in the amount of concentrate fed per gallon. There was a statistically significant difference between the two cow groups in the rate of increase in S.E. intake, but not in D.C.P. intake. In the group of higher yielding cows the rate of increase was 1·25 and in the other group 0·83 lb. S.E. per lb. increase in the amount of concentrates fed per gallon. Each additional pound of concentrates fed per gallon increased the concentration of S.E. in the diet as a whole by 2·2 units and of D.C.P. by 0·6 unit. There was a progressive increase in the overall concentration of the rations from 49 to 62 S.E. and from 7·6 to 11·4% D.C.P. from treatments A to D.

Table 3. *Nutrient intakes and levels of feeding*

	Consumption (lb./cow/day)		Level of feeding as % of Woodman's standards	
Treatment	S.E.	D.C.P.	S.E.	D.C.P.
A	11·6	1·8	97	89
B	13·5	2·3	109	110
C	15·4	2·8	118	124
D	17·8	3·3	131	142
Standard error of mean	±0·34	±0·08	±2·1	±2·3
Increase per additional lb. of concentrates fed per gallon (by regression)				
For all cows	1·03***	0·24***	5·6***	8·7***
Higher yielding group	1·25**	0·27***	5·5***	7·8***
Lower yielding group	0·83***	0·21***	5·7***	9·5***
Periods				
1	16·2	3·9	113	111
2	15·0	2·6	113	114
3	13·5	2·4	112	115
4	13·6	2·2	117	125
Standard error of mean	±0·34	±0·08	±2·1	±2·3
Coefficient of variation, %	6·5	8·5	5·3	5·7

The level of feeding of S.E. on treatments A to D respectively rose from 97 to 131% of the cow's requirements according to Woodman's standards (5), and that of D.C.P. from 89 to 142% (Table 3).

Animal production

Milk yield

The mean milk yields per cow per day for each 3-week period are included in Table 4. There was an increase from 22·2 lb. on ration A to 27·6 lb. on ration D and the differences between each of the treatments were statistically significant. There was also a significant

difference between the two groups of cows in their response to rising levels of concentrate feeding. For the higher yielding group the average yields from treatments A to D were 25·8, 28·0, 31·5 and 33·5 lb./cow/day, while in the lower yielding group they were 18·6, 19·6, 20·6 and 21·6 lb./cow/day. For each additional 1 lb. concentrate fed per gallon of milk, the increases in milk yield were 1·32 and 0·51 lb. for the higher and lower yielding groups of cows respectively. These increases were linear.

The average milk yields for each treatment during each 3-week experimental period were examined for the residual effects of the immediately preceding treatment by the method of Williams (2). The residual effects of treatments A and B were negative, and those of treatments C and D were positive, but all were small and non-significant.

Milk composition

The mean percentage composition of the milk for the two groups of cows is given in Table 4. The solids-not-fat (S.N.F.) content of the milk increased significantly with increasing levels of concentrate feeding. It was calculated by regression that for an increase of 1 lb. in the amount of concentrates fed per gallon (which Table 3 indicates was

Table 4. *Milk yields and composition*

Treatment	Milk yield (lb./cow/day)	Mean milk composition (g./100 g. milk)			
		Fat	Solids-not-fat	Crude protein	Lactose
A	22·2	3·99	8·34	3·06	4·37
B	23·8	3·94	8·40	3·09	4·36
C	26·0	3·99	8·58	3·20	4·47
D	27·6	4·09	8·60	3·27	4·44
Standard error of mean	±0·43	±0·08	±0·04	±0·03	±0·02
Increase per additional lb. of concentrates fed per gallon (by regression)					
For all cows	0·92***	0·02	0·05***	0·04***	0·02**
Higher yielding group	1·32*	0·04	0·08**	0·05**	0·03*
Lower yielding group	0·51	0·01	0·02	0·02*	0·01
Periods					
1	30·2	4·03	8·60	3·20	4·50
2	26·3	4·01	8·53	3·13	4·49
3	22·5	4·03	8·46	3·14	4·36
4	20·6	3·94	8·33	3·15	4·30
Standard error of mean	±0·43	±0·08	±0·04	±0·03	±0·02
Coefficient of variation, %	5·0	5·5	1·3	2·8	1·2

equivalent to an average increase of about 1 lb. S.E. per cow per day), the S.N.F. content increased by 0·05 g./100 g. milk. There was no significant difference between the cow groups in this respect despite the difference between them in the rate at which the S.E. intake increased. The S.N.F. contents declined significantly in successive periods. This was mainly the result of the normal fall in lactose content as lactation advanced and was accentuated in this experiment by two cows with shorter than normal lactations and one cow with sub-clinical mastitis. Any variations in fat content were not significant except between cows. The variations in S.N.F. were similar in all cows, although the greater response was shown in the milk of the higher yielders.

The increase in S.N.F. content of the milk on changing from ration A to ration B was

mainly in the casein fraction, while on rations C and D, which produced the higher yields, increased lactose content also contributed to the higher S.N.F. These results are similar to those found by Rowland (8) with cows on varying planes of nutrition. Most of the effect of a change of treatment on milk composition had apparently occurred when the milk was sampled at the end of the first experimental week of each period.

Live weights

The mean live weights of the cows on each treatment and in each period are given in Table 5. There was a progressive increase in the average live weight from 1054 lb. on treatment A to 1073 lb. on treatment D.

Table 5. *Live weights*

Treatment	Average live weight per cow (lb.)	Daily change during period (lb.)
A	1054	−0·37
B	1060	−0·12
C	1061	+0·12
D	1073	+0·67
Standard error of mean	±6	±0·22
Increase per additional lb. concentrates fed per gallon (by regression)		
For all cows	2·8*	0·20
Higher yielding group	4·6	0·26*
Lower yielding group	1·0	0·13
Periods		
1	1059	+0·92
2	1059	−0·22
3	1061	+0·03
4	1068	−0·04
Standard error of mean	±6	±0·22
Coefficient of variation, %	1·5	—

The linear regression coefficient was calculated for each cow on each treatment from the fifteen daily weighings made in each experimental period. Despite this high frequency of weighing, few of the individual regressions and none of the pooled regressions for each treatment reached significance. However, the coefficients showed a progressive change in daily gain from −0·37 lb. on treatment A to +0·67 lb./cow/day on treatment D. Within the higher yielding group the differences were greater. There was a loss of 0·62 lb./cow daily on treatment A and a gain of 0·95 lb. on treatment D. The mean increase in the rate of live-weight gain per lb. increase in the amount of concentrates fed per gallon of milk was only 0·20 lb./cow/day and this was non-significant.

DISCUSSION

Experiments reported by a number of workers (9,10,11) suggest that with milk yield the law of diminishing returns operates at normal levels of feeding. However, Brody (12) has suggested that a straight line might equally well have been fitted to the data of Jensen *et al.* (9) and very high efficiency of feed conversion has been found with cows yielding as much as 1000 lb. fat in a lactation (13).

In an earlier experiment in this series (14) the milk yield and milk composition responses showed diminishing returns with increased feed input, although the total production as milk plus live-weight gain showed a rectilinear response. In the present experiment, the increments in milk yield with each rise in the rate of concentrate feeding were on average equal. The difference between these two results is probably due to the ration of the highest nutritive value in the earlier experiment having provided a level of feeding considerably above that of the highest in the present experiment. The results reported here show that in mid-lactation and with basal feed of the moderate qualities used, each additional 1 lb. of S.E. input was rewarded over a fairly wide range of feeding levels by the additional production of 0·9 lb. milk and an increase of 0·05 in the percentage of S.N.F. in the milk.

This clear relation between the level of feeding and that of the milk S.N.F. was not found in the previous experiment (14), and the difference was again probably due to the lower general level of feeding in the present experiment. The general level of S.N.F. on all the rations in this experiment was low, particularly in the milk of the higher yielding cows. At comparable stages of lactation, the milk of well-fed Ayrshire cows might have been expected to contain about 8·8% S.N.F. with 3·2% crude protein (4). Thus the protein content was below normal on treatments A and B, but rose to normal on treatments C and D. This suggests that the cows were underfed in treatments A and B despite B being theoretically above and A very near the Woodman standard. This raises the question, therefore, whether the nutrient values assumed for the grass feeds, although reasonable, were not too high. There is growing evidence that cows fed rations high in silage (see, for example, (15)) produce milk low in S.N.F. content and the present results appear to confirm this. It may be that on a diet consisting largely of low or medium quality bulk foods, high-yielding cows cannot obtain sufficient nutrients for their requirements. If this were so, both the yield and the quality of the milk would suffer, as they did in this experiment.

Without concentrates the total daily consumption per cow of dry matter in the form of hay, silage and dried grass was equivalent to only 2·24% of the cow's live weight. This is about the same dry-matter consumption as that shown by cows fold-grazed on herbage at the long leafy stage (16), but owing to the losses involved in grass conservation and to the more advanced stage of growth of the herbage when conserved, this rate of dry-matter consumption is evidently inadequate in winter feeding. It has been pointed out on theoretical grounds that in winter feeding the concentration of the diet must be raised to meet the needs of high-yielding cows (17, 18). The influence of additional concentrates in increasing both the total dry-matter intake and the concentration of the diet is shown clearly by the results of the present experiment.

When the profit margins per day of the four treatments were compared, it was found that on average the highest level of concentrate feeding compared with the lowest gave a reduction in profit margin of about 8*d.* per cow per day, but that in the group of higher yielding cows there was no consistent difference in profit margin between the treatments. The short duration of the experimental periods must be taken into account here, however. Whatever the profit margin in the short-term, it is unlikely that the lower levels of rationing would have been desirable for a prolonged period. Not only was there a decline in live weight and milk quality, but treatment A at least would have resulted in an abnormally rapid decline in milk yield and probably a shortened lactation. This was also found by Jensen *et al.* (9) with cows fed only roughages. On the other hand, treatment D

would probably have resulted in an unnecessary increase in live weight. It appears, therefore, that for the maintenance of live weight and milk quality, concentrate feeding at the level of treatment C, 4 lb./gal., was best in the conditions of this experiment. Of course, if the silage had been higher in quality, a lower concentrate rate might have sufficed, and vice versa. It may be noted that in a preceding experiment(1) in which more dried grass was fed in addition to silage, 2 lb. concentrates per gallon gave similar results to those obtained with 4 lb./gal. in the present experiment.

SUMMARY

1. An experiment is described which assessed the effects on the production obtained from milking cows, of feeding a concentrate with a starch equivalent of 64 at four levels (0, 2, 4 and 6 lb./gal. of milk produced), in addition to a basal ration with limited amounts of hay and dried grass and with silage fed *ad lib.*

2. The total daily dry-matter intake of the cows increased from 2·2 to 2·7% of the live weight as the rate of concentrate feeding was increased from 0 to 6 lb./gal., despite a reduction in the amount of roughages consumed.

3. For each increase of 1 unit in silage dry-matter percentage, there were increases of from 1·18 to 0·42 lb. of silage dry-matter intake per cow per day as the level of concentrate feeding increased. For each increase of 1 lb. in silage dry-matter intake there was an increase of 1 lb. in total dry-matter intake per cow per day.

4. The average daily milk yield per cow increased from 22·2 lb. when no concentrates were fed to 27·6 lb. when 6 lb. concentrates were fed per gallon of milk produced. This is an increase of 0·9 lb. milk/lb. increase in starch equivalent intake. A significant difference in response was found between higher yielding and lower yielding groups of cows. The higher yielding group gave an increase of 1·1 lb. and the lower yielding group only 0·6 lb. milk/lb. increase in starch-equivalent intake.

5. At the general level of feeding and of milk yields obtained in this experiment, no evidence of diminishing returns in milk production was detected when the rate of concentrate feeding was increased.

6. The content of solids-not-fat in the milk rapidly increased from 8·3% when no concentrates were fed to 8·6% when 4 or 6 lb. of concentrates were fed per gallon of milk produced.

7. When concentrates were fed at the rate of 0 and 2 lb./gal. declines in live weight as well as in the yield and solids-not-fat content of the milk were noted, while at 6 lb./gal. there was an unnecessary gain in live weight. It is concluded, therefore, that with the particular animals and roughages used in this experiment, the optimal level of concentrate feeding was 4 lb./gal.

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648. WEIGHT CHANGES IN GRAZING COWS

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(With 4 Figures)

It has frequently been observed that when cattle are changed from winter feeding to grazing there is a temporary loss in weight. Although perhaps not a serious matter for farmers, this loss can be a source of considerable error in experimental attempts to measure the nutritive value of grazing, since in such experiments it is nowadays usual to equate the value of the pasture with the growth or production of the experimental animals. Even where milk is the major product it is not advisable to ignore live-weight changes, although this is often unavoidable. Because the losses may be in excess of 5% and occur rapidly, often within the first 3 or 4 days after the beginning of grazing, it has been widely concluded that they were unlikely to be due entirely to loss of body substance, but were probably due mainly to a fall in the weight of the gut contents. Reports on this problem were reviewed by Tayler(1), who studied the bias that might be introduced into estimates of the carcass gain of bullocks due apparently to changes in gut fill resulting from the season of the year, herbage quantity and quality, and grazing management. In sheep and bullocks, fasting for 24 or 36 hr. was effective in reducing certain of the differences in weight due to 'fill'. 'Residual fill', measured in slaughtered sheep by weighing the viscera and their contents after 24 hr. of fast, showed a relationship with the live weight. In fistulated cows receiving freshly cut herbage indoors, losses of 30–35 lb. in the contents of the reticulo-rumen have been reported(2).

In fistulated cows the entire contents of the reticulo-rumen can be readily removed, weighed, sampled and returned without disturbance to the animal. This communication presents data on losses in weight during grazing that have been collected by this means from cattle in the Institute herd. These data are of two types. First there are summaries, extending over two grazing seasons, of the weights of the contents of the reticulo-rumen of seven adult fistulated cows in two experiments, values for the same cows receiving winter diets being given for comparison when available. Secondly, a description is given of a third experiment designed to demonstrate the proportion of the weight loss due to changes in the contents of the reticulo-rumen. Mäkelä(3) observed that in twenty-four cows slaughtered while receiving intakes of hay ranging from 3·0 to 13·0 kg. daily the contents of the reticulo-rumen formed 67–85, mean 74%, of the total gut contents and the proportion changed little with changing level of intake. It seemed likely therefore that the weight of the contents of the reticulo-rumen would be closely correlated with the weight of the contents of the whole gut.

METHODS

Experiments 1 *and* 2. Details of the diets received by the various fistulated cows are given with the results in Tables 1 and 2. The term 'restricted grazing' is used in these tables to show that the cows were allowed strip-grazing by means of an electric fence. The

amount of grass offered was always considered ample for an average milking cow. The cereals grazed by cows D. 29, R. 37 and W. 26 consisted of a mixture of oats and barley at an advanced stage of growth and showing ears for most of the grazing period; this material was considerably more fibrous than the grass swards.

Experiment 3. This experiment involved thirty-eight Friesian and Shorthorn cows, yielding on average 31 lb. milk daily at the start of the experiment, and three fistulated cows of which two, D. 15 and L. 32, were in milk while the third, K, was dry. In an unrelated experiment, to be reported separately, observations were made on the blood composition of the non-fistulated cows. The fistulated cows used in these experiments were all Shorthorns. During the winter months sixteen of the non-fistulated cows received daily approximately 8 lb. hay, 40 lb. ensiled brewers' grains and concentrates in proportion to the yield in excess of 10 lb. milk; eleven cows received 7 lb. hay and 32 lb. brewers' grains and concentrates according to yield. The fistulated cows began grazing after the evening milking on 24 April and the remainder at the same time on the previous day. For the purposes of the experiment it was thought desirable to make the change to pasture an abrupt change.

Two similar adjacent fields, totalling 22 acres, were used for the experimental grazing. A ley consisting of meadow fescue (*Festuca pratense*) (S 53), timothy (*Phleum pratense*) (S 48), white clover (*Trifolium repens*) (S 100 and S 184) was established in the previous spring. Botanical analyses, by the method of species contribution in the dry matter of the herbage on offer, indicated that the sown species contributed 80–90% of the total, the remainder consisting largely of annual weeds. The weeds were to be expected in the initial crop of a ley of this type in its first harvest year. The sward was typically rather open and the contribution of the clover was small, due mainly to the frosts occurring at this time.

The cows were strip-grazed by means of electric fencing to allow adequate herbage without overfeeding. The fresh allocation of herbage was given following the afternoon milking. Occasionally the ration appeared inadequate and in such cases a further allocation was made the following morning. Small supplements of various concentrates were given, mainly during the afternoon milking, consisting of 0, 2 or 4 lb. daily per cow. These treatments were extraneous to this experiment and examination of results failed to indicate that they had any effect on the weights of the cows.

Results of measurements, by a method of pre-grazing and residue sampling (Line—unpublished), indicated that the average weight of dry matter in this sward was 1720 lb./acre, of which 817 lb. was utilized. Considering the amount of herbage on offer, this represents reasonably hard grazing.

The non-fistulated cows were weighed daily on a weighbridge accurate to ± 2 lb., from 17 April–15 May at 07.30–08.00 hr. and the fistulated cows with them after they began grazing. In addition, the fistulated cows were weighed at 11.00–13.00 hr. immediately before each emptying of the reticulo-rumen.

Removal of the contents of the reticulo-rumen

The rumen fistulas were closed by means of rubber cannulas and bungs and removal of the contents by hand through the opened cannula could be readily accomplished, a jar being necessary only for the last small amounts of fluid digesta. The entire contents were removed, weighed, sampled and returned in an average time of 30 min. The cows were

examined in the same order on each occasion. Representative samples were taken from each of the three to five large pails required for the digesta and dried to constant weight at 100° C. It is estimated that the weight of the contents of the reticulo-rumen can be determined within an error of −0·5 lb.

RESULTS

Experiment 1. The results summarized in Table 1 show that when consuming 18 lb. hay daily the reticulo-rumen of cow W. 22 contained 165·5 ± 6·6 lb. digesta, but when given free access to a perennial ryegrass and white clover ley, showing good autumn growth, the contents fell to 129·8 ± 12·4 lb. The weight of dry matter showed a proportionally larger fall, since the dry-matter percentage also fell. Values for cow D. 15 showed similar trends, although the winter diets consisted, at different times, of hay *ad lib.*, 18 lb. hay and 8 lb. concentrates, 16 lb. hay and 20 lb. concentrates, or 16 lb. hay and 16 lb. concentrates.

Table 1. *Exp.* 1. *Contents of the reticulo-rumen (mean and standard deviation) of cows D.* 15 *and W.* 22 *on winter feeding and grazing*

Cow	Date*	Diet	Contents of reticulo-rumen: Total (lb.)	Dry matter (lb.)	Dry matter (%)
D. 15	Aug. 1951–July 1952 (8)	Various, hay + concentrates	156·2 ± 11·9	19·2 ± 3·8	12·2 ± 1·5
	Sept.–Oct. 1951 (7)	Grazing ample ley, unrestricted	128·8 ± 14·7	10·8 ± 2·0	8·4 ± 1·2
W. 22	Aug. 1951 (7)	18 lb. hay	165·5 ± 6·6	17·5 ± 2·0	10·6 ± 1·1
	Sept.–Oct. 1951 (8)	Grazing ample ley, unrestricted	129·8 ± 12·4	12·1 ± 2·0	9·2 ± 0·7

* Values in parentheses show number of observations.

Table 2. *Exp.* 2. *Weight of total contents and of dry matter in reticulo-rumen of six cows receiving various diets*, 1955

Diet	Date	Total (lb.)	Dry matter (lb.)	Date	Total (lb.)	Dry matter (lb.)	Date	Total (lb.)	Dry matter (lb.)
	Cow D. 15			Cow K			Cow L. 32		
14 lb. hay, 5 lb. dried sugar-beet pulp	9 Mar.	176·1	20·2	9 Mar.	168·7	17·1	9 Mar.	178·4	18·6
	11 Mar.	179·6	20·4	11 Mar.	175·2	17·6	11 Mar.	186·3	19·2
	6 Apr.	168·9	21·1	6 Apr.	160·3	15·5	6 Apr.	156·2	15·0
Unrestricted grazing of rather sparse old pasture from 27 Apr.	7 June	114·3	12·0	7 June	135·1	14·8	9 June	111·5	9·0
	13 June	113·8	13·1	13 June	130·4	14·1	16 June	101·2	8·4
	17 June	108·3	10·8	17 June	124·9	13·3	21 June	114·0	9·1
	20 June	105·5	10·8	20 June	124·7	12·6	27 June	117·6	8·9
							30 June	114·7	7·5
							23 Aug.	121·2	12·6
	Cow D. 29 (grazing from 20 Apr.)			Cow R. 37 (grazing from 12 May)			Cow W. 26 (grazing from 12 May)		
Restricted grazing, grass	8 June	140·5	16·3	8 June	132·1	14·6	8 June	125·3	11·6
Ample grazing, grass	10 June	158·0	19·6	10 June	156·5	19·1	10 June	148·8	16·8
Ample grazing, cereals	15 June	156·4	18·7	15 June	170·2	19·5	15 June	169·5	15·8
Ample grazing, cereals	22 June	209·7	26·6	22 June	188·7	23·4	22 June	193·1	21·2
Ample grazing, cereals	23 June	164·0	20·5	23 June	169·2	19·9	23 June	168·5	18·1
Ample grazing, cereals	28 June	165·6	18·0	28 June	170·4	19·5	28 June	166·9	15·2
Ample grazing, cereals	20 July	186·1	21·9	20 July	162·5	19·2	—	—	—
Sparser grazing + lucerne for 2 hr. in morning	26 Aug.	176·6	28·4	23 Aug.	157·5	15·2	26 Aug.	184·2	22·6

Experiment 2. Observations on the six cows are given in Table 2. With a winter diet consisting only of 14 lb. hay and 5 lb. sugar-beet pulp the total contents of the reticulo-

rumen of the dry cows D. 15, K and L. 32 varied from 156 to 186 lb. and the dry matte from 15 to 21 lb. The amounts found when they were established on a rather sparse o pasture, to which they had ample access (four cows, one non-experimental, on nine acre and which would in practice have been considered more than sufficient for four dry cows, were usually at least 50 lb. lower than with the winter diets. The weight of dry matter fell by about 50% in cows D. 15 and L. 32, but less markedly in cow K.

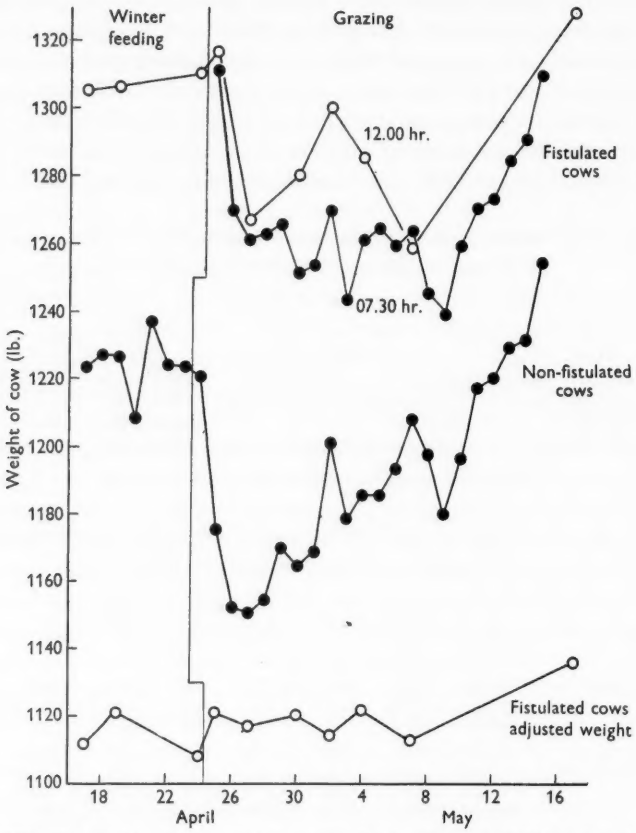

Fig. 1. Exp. 3. 1956. The weights of the fistulated and non-fistulated cows at 07.30 hr., ●, and of the fistulated cows at 12.00 hr., ○, with winter feeding and grazing. The adjusted weight of the fistulated cows was obtained by making allowance for the estimated contents of the digestive tract (see p. 16).

In the three cows in milk, D. 29, R. 37 and W. 26, for which no values on winter diets were available, there was a tendency for values found on 8 and 10 June to be lower than would be expected with a winter diet, but when the cows were grazing green cereals amounts of digesta were found in the reticulo-rumen similar to those found with winter feeding in the other cows. This applied also to the weight of dry matter.

Experiment 3. The effect of the change from winter feeding to grazing on the average weight of the cows is shown in Fig. 1. On the third, fourth and fifth days after the change in diet the average fall, and the standard deviation of the falls in individual non-fistulated

cows, was 71 ± 24 lb. Following the low point on the fourth day, the weights increased fairly regularly throughout the remaining 18 days of observation, the regression for these weights indicating an average daily gain of 4·5 lb. per cow. At the end of the period the mean weight was greater than that found on winter feeding. Longer observation was not possible as all the grass in the field had been used.

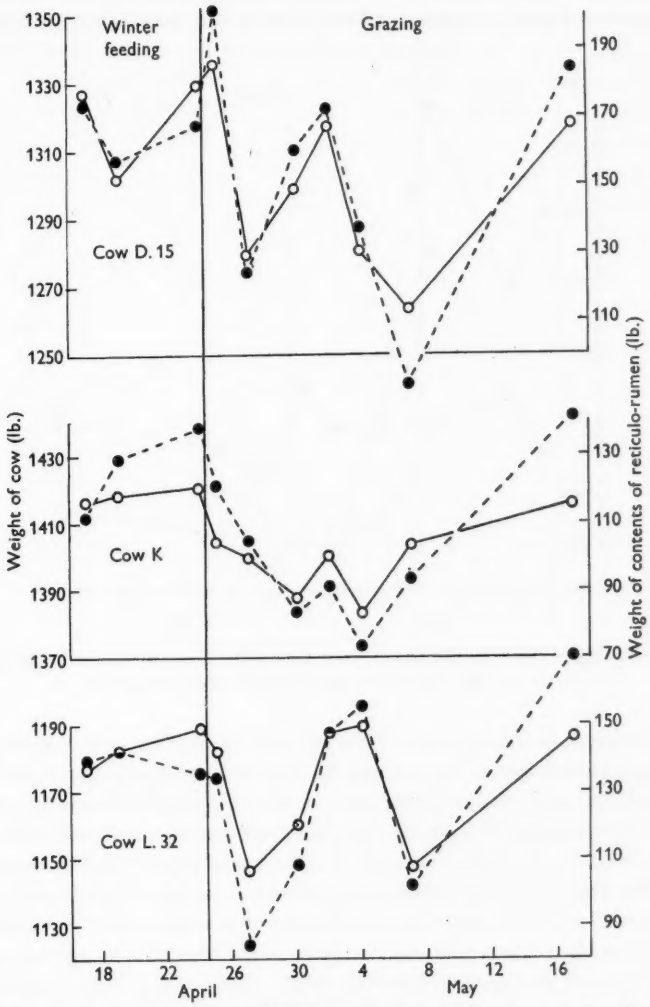

Fig. 2. Exp. 3. The weight of cows D. 15, K and L. 32 immediately before emptying in Exp. 3, ●--●; with the weight of the contents of the reticulo-rumen, ○—○.

The weights of the fistulated cows also fell after the beginning of grazing, although the pattern of fall and recovery was less regular than in the other cows. The divergence of the weights at 07.30 and 12.00 hr. on 2 May might have been due to less restriction of grazing, allowing a larger proportion of the daily intake to be consumed during the morning than

in other parts of the experiment; by 17 June the weights had regained the levels found with winter feeding. The marked similarity between the response of the fistulated and non-fistulated cows permits the assumption that changes in the weight of the reticulo-ruminal contents would follow the same trends in each group.

In Fig. 2 the weights of the digesta in the reticulo-rumen of each fistulated cow are shown plotted beside the weights of the cow. It is evident that both the small day-to-day and the larger treatment-to-treatment variations in the weights of the cows were due

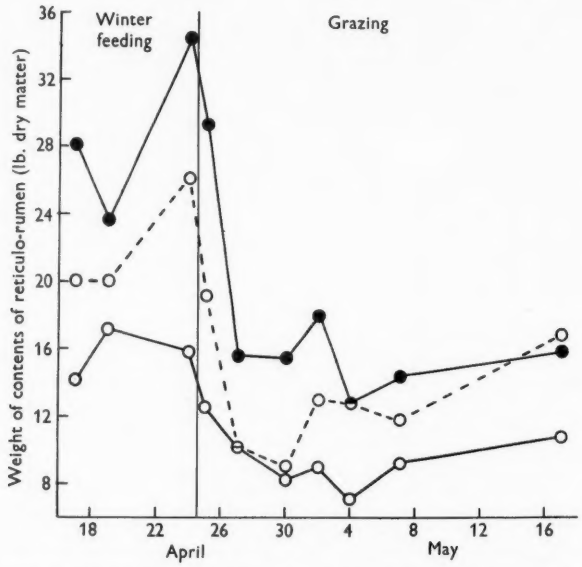

Fig. 3. Exp. 3. The weight of dry matter in the reticulo-rumen of cows D. 15, ●—●, K, ○—○, and L. 32, ○- -○, with winter feeding and grazing.

mainly to variations in the contents of the reticulo-rumen. The regression coefficients of weight changes on changes in the amount of digesta were significant at the 0·1% level (D.F. = 8), and this analysis indicated that, of the variation in the weight of the cows, variation in the weight of the reticulo-ruminal contents was responsible for 88, 83 and 81% (mean 84%) in cows D. 15, K and L. 32 respectively. If it is assumed that the contents of the reticulo-rumen formed 74% of the contents of the whole gut (see p. 11), the calculation of multiple regression coefficients shows that when allowance was made for changes other than in gut contents (i.e. growth or fattening) the regressions of weight changes on changes in this hypothetical value were $0{\cdot}958 \pm 0{\cdot}125$, $1{\cdot}289 \pm 0{\cdot}224$ and $1{\cdot}046 \pm 0{\cdot}131$ in cows D. 15, K and L. 32 respectively. These values were significant at the 0·1% level (D.F. = 7). The same analysis indicated that when changes in gut content were eliminated cows K and L. 32 gained $0{\cdot}392 \pm 0{\cdot}137$ and $0{\cdot}815 \pm 0{\cdot}340$ lb. daily throughout the experiment, these being significant only at the 20% level. When presented graphically (Fig. 1) the values for the live weight adjusted for the gut contents (= contents of reticulo-rumen × 100/74) show no change in the first 2 weeks of grazing, but there may have been an average gain of about 23 lb. in the period 7–17 May.

The amounts of dry matter in the reticulo-rumen are shown in Fig. 3 to have declined to a proportionally greater extent than did the total weight of the contents and showed only a slight tendency to recover towards the end of the period of observations. With winter feeding the reticulo-rumen contained 28·7, 15·7 and 22·1 lb. dry matter in cows D. 15, K and L. 32; during the period 30 April–7 May, when the effect of grazing appeared to be maximal, comparable values were 15·1, 8·3 and 11·6 lb. This suggests that in the recovery in the weight of contents observed at the end of the period, increases in water tended to occur without parallel increases in dry matter.

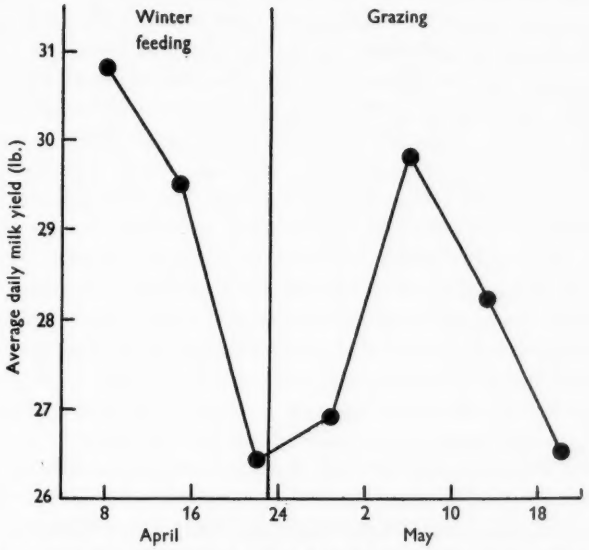

Fig. 4. Exp. 3. The average daily milk yield of the thirty-eight non-fistulated cows, with winter feeding and grazing.

The milk yields of the non-fistulated cows (Fig. 4) responded to the change in diet by showing a reversal of the lactation decline as is normal when cows are turned out to grass. During the week ending 6 May only six of the thirty-eight cows gave less milk than in the week ending 22 April. As far as trends could be discerned the increases in yield appeared to be greatest in the highest yielders. Since some cows were giving 45 lb. daily on 22 April the milk yields suggest that the amount of grass allowed to the cows would be considered adequate under normal management.

DISCUSSION

With rationing of grazing to an extent that might prevail on a well-managed dairy farm thirty-eight cows averaging 1124 lb. in weight lost an average of 71 lb. or 6·3% in the first few days of the change to grass. In the six most extreme cases the figures were over 100 lb. or 8·4% of their initial weight. From the considerable informal discussion that has taken place on this subject in this country in the past year or two it is evident that these falls are not exceptional, although in Exp. 3 the change in diet was more sudden than

would be expected under good management. It would, however, be wrong to suppose that these falls are necessarily typical of all cattle under British conditions, or that the average falls observed here are in any way generally applicable. It would seem likely that conditions will be found where, on going out to grass, groups of cows will lose more weight than we have recorded and that under other conditions there may be no weight loss or it may be much less. Where, however, such losses occur, it is evident from the results obtained with the fistulated cows, that they are due very largely to changes in the weight of digesta in the reticulo-rumen. If, as seems likely from the data of Mäkelä (p. 11), the contents of the remainder of the gut varied in the same proportion as the contents of the reticulo-rumen, it would appear that these short-term changes in weight were probably due almost entirely to changes in the content of the whole gut, any residual variation being explicable by differences in the fill of the bladder. This was strikingly demonstrated when the weights of the fistulated cows were corrected for gut contents (Fig. 1). This adjustment completely eliminated the substantial loss of weight shown by all cows after the commencement of grazing. It is also likely that in our experiments there were some few pounds of true growth during the course of the observations.

A further possibility to be considered is whether, especially in view of the increased intake and excretion of water in cows receiving grass, there is any loss of tissue water at the time of the changes in diet. Our observations on cows receiving grass indoors, either freshly cut or stored frozen, rarely show any negative water balance (this value does not account for expired water vapour) and no more reduction in the usual positive figure than would necessarily accompany a reduction in the amount of water in the gut contents (2). Although this is far from conclusive evidence, it seems unlikely that tissue dehydration plays any part in weight losses at the commencement of grazing.

Realization of the magnitude of the losses resulting from reductions in gut fill emphasizes the need for great care in the interpretation of the results of grazing trials. Thus if, in Exp. 3 of our investigation, observations had been begun only 4 days after the change in diet, it might have been concluded that during the next 18 days the animals were growing or fattening at a mean rate of 4·5 lb. daily, whereas it is unlikely, judging by the fistulated cows, that their empty body weight changed by as much as 0·6 lb. daily in the whole period of the experiment. The experimenter is accordingly faced with the need for a procedure which will avoid this difficulty. With foods that can be stored it is traditionally avoided by the use of preparatory periods in which the amount of the gut contents become stabilized. There seems little chance of the wide use of this sound procedure in grazing investigations.

In studies with non-lactating cattle, fasting for 12 hr. or more to reduce the effects of variations in gut fill is a widely accepted practice (1). Although this appears to be a useful technique for experiments with growing or fattening animals, it is doubtful if it is satisfactory for experiments with lactating cows, and the results here reported throw no light on its effects.

Information given and quoted by Mäkelä (3) suggests that the physical state is of greater importance than the level of intake in determining the amount of digesta present in the gut, and it was particularly surprising to find that in his experiments with the diets ranging from 3 to 13 kg. hay daily the total amounts of digesta ranged only from 57 to 117 kg. At the lower levels the gut tended to contain a higher proportion of water. The prediction of the amount of gut contents at any given stage in a grazing trial will therefore

be influenced by such factors as the physical fibrousness of the sward as well as by the amount available, the appetite of the animal and probably, in the early stages of the trial, by the previous diet. The changing nature of these factors renders this a most uncertain prediction.

The difficulty of predicting the amount of gut contents would be avoided if it could be shown that there were some 'normal' or optimum gut content for every cow to which the amounts would become adjusted in a set time, e.g. 2 or 3 weeks, after changes in diet. With winter diets, containing reasonable quantities of roughages in the long state, the errors introduced by this assumption will be small, but with diets containing grass, small amounts of roughage or roughage in the ground state they may be more serious. Thus, on some of the swards used in our experiments the amounts of gut contents appear to have fallen initially and recovered in about 3 weeks, whereas with other swards a low level of gut contents persisted. With cows grazing fairly fibrous green cereals amounts were found equal to or even in excess of these to be expected with winter feeding. It is hardly to be doubted that with an ample, but fairly mature, grass sward similar amounts of gut contents might eventually be found, following some initial loss.

There has long been a need, in nutritional experiments, for a means of determining the true rate of body increase in ruminants by some technique which eliminates the effect of gut fill and yet causes no disturbance to the animal. As discussed by Reid, Wellington & Dunn (4) the use of dilution techniques, with markers such as antipyrene, for the determination of body water appears to point the way to a system which might be developed for this purpose.

SUMMARY

1. Under various feeding regimes the entire contents of the reticulo-rumen of fistulated Shorthorn cows were repeatedly removed, weighed, sampled and returned.

2. With cattle grazing grass/clover swards, but not with others grazing green cereals, there was a very marked tendency for less digesta to be found in the reticulo-rumen than with several winter diets.

3. An average fall of 71 ± 24 lb. was found in the weight of a mixed herd of thirty-eight Friesian and Shorthorn cows when they were changed suddenly from winter diets to grazing. Simultaneous observations on three fistulated cows showed that 84% of such short-term variation in weight could be explained by variation in the weight of the digesta in the reticulo-rumen. It is likely that smaller, parallel variations in the weight of the contents of the remainder of the gut accounted for most of the remaining differences.

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649. THE EFFECT OF DESIGN OF TEAT-CUP LINERS ON MASTITIS INCIDENCE

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(With 2 Figures)

INTRODUCTION

The primary physical forces applied in machine milking which affect the removal of milk from the udder are the vacuum of the milk line and the pulsation. As both of these forces are applied to the teat in the rubber teat-cup liner, it is to be expected that variation in liner design will influence the efficiency of milking and this, in turn, may influence udder health. Recent research has, in fact, demonstrated that the design of the liner influences both the rate of milking and the proportion of milk which is removed at the end of milking by machine stripping (1).

From the results of a number of herd surveys and field experiments it has been claimed that teat-cup liner design affects mastitis. A high incidence of mastitis in dairy herds has been said to be associated with some types of moulded teat-cup liner by Leslie & Whittlestone (2,3), Watts (4,5), Gambrel (6), N.I.R.D. Annual Report (7), Neave, Phillips & Mattick (8), Wilson (9,10) and Parker (11). On the other hand, Ward, Whittlestone, Hopkirk & Filmer (12), Ward (13) and Whittlestone (14) could find no difference in the incidence of mastitis in herds using a particularly hard moulded liner compared with herds using liners of a different design. A number of cases have been cited (3)–(11), where a change from moulded liners has been followed by a reduced herd incidence of mastitis within several months, and experiments by Wilson (9) confirmed this.

In Britain teat-cup liners can be classified into two groups according to whether they have been extruded or moulded in manufacture. The former are extruded as rubber tube, which is cut to length and then given the characteristic liner shape when the mouthpiece is formed by inserting a metal ring at one end (Fig. 1). Moulded liners, as their name suggests, are rubber castings of the liner in its final shape. All liners differ in other ways—particularly in the diameter and design of the mouthpiece and in the internal diameter of the barrel. One of the main differences lies in the load necessary to elongate the liner from rest to the milking position; this elongation may vary from less than 5% to about 20%.

Previous data (8), when extracted (7), showed that there was very significantly more mastitis in both of two herds when they were milked with a moulded liner than when milked with an extruded liner. The experiment reported below was designed to see if this result could be confirmed under more carefully controlled conditions and when the two

liners were used simultaneously on the same udder. Furthermore, we wished to know if there was an influence on the number of new infections as well as on mastitis and whether or not milk yield, leucocyte count and teat erosion were affected.

THE HERD

The experiment was carried out in the herd of the National Institute for Research in Dairying from April 1953 to September 1954. The herd and its general management and milking routine have been described (15) and all animals in milk during the period were included in the experiment.

EXPERIMENTAL METHODS

The dimensions of the liner are shown in Fig. 1. The moulded liner had a hooded mouthpiece and a barrel connected to the short milk tube in manufacture, the barrel being at a relatively low tension in the milking position. The extruded liner was not permanently attached to the short milk tube, and was held at a high tension when in position in the teat cup.

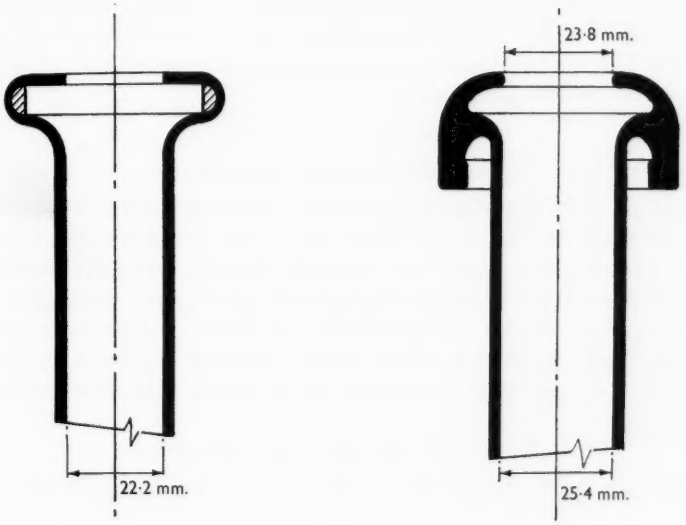

Fig. 1. The design of the liners used in the experiment. Dimensions are for new liners. Average mouthpiece diameters in the field are: extruded, 21 ± 3 mm., and moulded, 24 ± 1 mm.

The standard milking machine used was a bucket type operating at a pipe-line vacuum of approximately 15 in. Hg, a pulsation rate of 50 cyc./min. and a pulsation ratio of 1:1. Composite clusters of moulded and extruded liners were used so that the experimental comparison could be made more precisely on a within-cow basis. The two quarters on the right side of the udder were milked with moulded liners and those on the left with extruded liners. The weight of the teat-cups was adjusted so that the total weight of the teat-cup assembly was approximately normal for each design (i.e. $5\frac{1}{2}$ lb.).

The care of teat-cup liners

Two sets of liners were provided and used alternately each 14 days. Liners not in use were immersed in a 5% solution of caustic soda to remove most of the milk and body fat adsorbed during milking. After removal from the caustic soda solution the liners were brushed to remove all deposits, examined and all faulty items replaced. The liners were thus kept in good condition.

After milking, the liners were brushed in cold water and hot alkaline detergent before rinsing in warm water. After the morning milking the liners were steamed under tension for 3 min. and in the evening they were rinsed in a cold solution of sodium hypochlorite (150 p.p.m. available chlorine).

Method of estimating effect of experimental routine on milk yield

Cows that were not infected on calving or infected in not more than two quarters (one in each udder half) were milked with an individual quarter bucket milking machine on two occasions during lactation, i.e. when maximum daily yield was reached (at about the sixth week of lactation) and again when daily milk yield had fallen to half of this maximum. The mean stage of lactation when the second quarter milkings took place was 24 ± 8 weeks after calving. On each occasion the records were taken at four consecutive milkings. The data were analysed to show if the decline in yield with advance in lactation was different for the two udder halves.

BACTERIOLOGICAL METHODS

The methods by which the udder health records were collected and assessed have been described previously (15). Leucocyte counts were made on quarter milk samples at the time when quarter milking was done. Standard 1 cm. square films were prepared in duplicate, stained with carbol thionin and examined by one person using an oil-immersion lens and an eyepiece with an aperture 8·5 mm. square (16). For the purposes of analysis the leucocyte count was the sum of the number of leucocytes seen in a strip across the centre of each of the two films. Each strip was the length of the film (left to right) and 0·13 mm. wide.

Therapeutic treatment of infected quarters

Treatment was confined, with few exceptions, to quarters affected with mastitis.

RESULTS

Records were available from 84 cows that had a mean age of 3·8 lactations.

The incidence of previous infection, i.e. infection that was established before the experiment began, new infection and mastitis is shown in Table 1. From the summary of the udder health data given in Table 1 it will be seen that there was a higher incidence of mastitis in the quarters milked with the moulded liner. Of these quarters 12·7% showed signs of mastitis compared with only 5·5% of those milked with the extruded liner, the difference being significant ($P < 0{\cdot}05$). Where the comparison was confined to mastitis in infected quarters the difference between the groups was highly significant ($P < 0{\cdot}01$). There was no difference in the incidence of new infection in quarters that were free from

infection at the start of the experiment. The incidence of mastitis was similar for these newly infected quarters, but the numbers were small.

Table 1. *The number of quarters in milk, the number infected, the type of infection and the number of quarters affected with mastitis according to type of teat-cup liner in use*

	Moulded liner						Extruded liner					
	Quarters			*Type of micro-organism			Quarters			*Type of micro-organism		
	No.	†Clin.	%	S	III	O	No.	Clin.	%	S	III	O
No. of quarters in milk	165	21	12·7	—	—	—	165	9	5·5	—	—	—
No. of quarters excluded‡	3	—	—	—	—	—	3	—	—	—	—	—
No. of quarters with previous infection§	46	17	37·0	38	5	3	51	5	9·8	44	6	1
Percentage	—	—	—	82·6	10·9	6·5	—	—	—	86·3	11·8	1·9
No. quarters free from infection at calving	119	—	—	—	—	—	114	—	—	—	—	—
No. quarters free from infection at calving and becoming infected	18	4	22·2	12	5	1	22	4	18·2	15	6	1
Percentage	—	—	—	66·7	27·8	5·5	—	—	—	68·2	27·3	4·5
Total no. infected quarters	64	21	32·8	50	10	4	73	9	12·3	59	12	2
Percentage	—	—	—	78·1	15·6	6·3	—	—	—	80·8	16·5	2·7

* S = *Staphylococcus aureus*; III = *Streptococcus uberis*; O = other micro-organisms or mixed infections.
† Clin. = mastitis (15).
‡ Five trodden teats and one non-functional quarter excluded.
§ Infection established before experiment began.

Effect on teat condition

The condition of the teat orifices was examined before the experiment began and on five subsequent occasions. The proportion of each group showing teat orifice erosion at each examination is shown in Fig. 2. It is clear that there were no marked differences in the effect of these two liners on teat orifice erosion.

Leucocyte content of milk

Of the cows milked with the quarter milking machine, fourteen were not infected in any quarter during the lactation in which these measurements were made. The leucocyte count of the milk of the twenty-eight quarters of these cows milked with the moulded liner increased by 314% between the first and second measurements of the individual quarter yields, while the count of the twenty-eight quarters milked with the extruded liners increased by 308% (Table 2). This difference is very small and not statistically significant.

Table 2. *The leucocyte counts* of milk from fourteen cows that were not infected, classified according to type of teat-cup liner in use*

	Total leucocyte count		
Total counts of one side of udder	Quarters milked with moulded liners	Quarters milked with extruded liners	Total
1st count	568·0	697·0	1265·0
2nd count	1785·0	2144·0	3929·0
Percentage increase	314·3	307·6	310·6

* These figures should be multiplied by $\frac{7700}{2 \times 14}$ to give the absolute number of leucocytes per ml.

The milk yield of individual quarters

Records of the yields of the individual quarters were obtained at two stages of lactation from thirty-seven cows of which fourteen were not infected during the lactations examined. Of these cows, twenty-seven gave a lower proportion of their milk yields in the quarters milked by the moulded liners at the second test than at the first. At the first test the mean

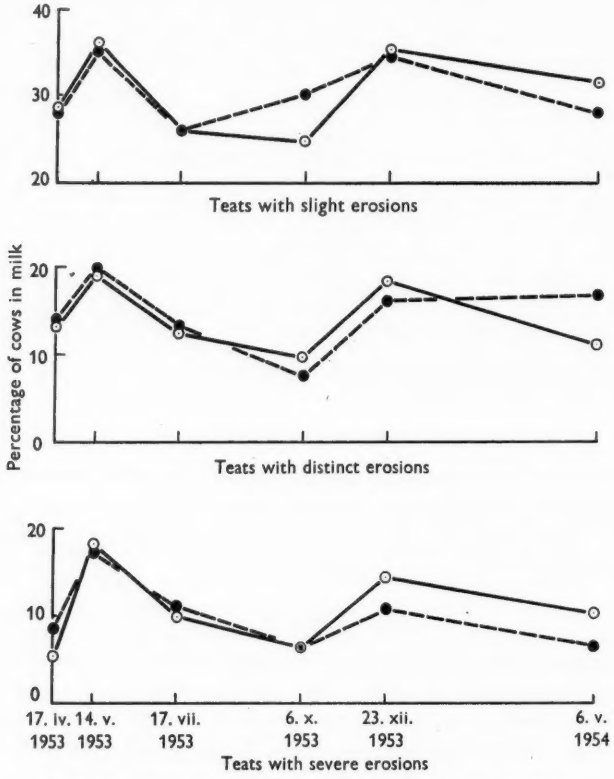

Fig. 2. The effect of type of teat-cup liner on teat orifice condition.
●, extruded liner; ⊙, moulded liner.

proportion of the cows' milk yield obtained from the RF and RH quarters of the thirty-seven cows was 49·6 %, and at the second 48 %. This decline of 1·60 ± 1·06 is not significant ($P < 0{\cdot}05$), but suggests that a real difference might be demonstrated if the results from more cows had been available. The results of the fourteen non-infected cows were similarly not significant, the proportionate yields of the RF and RH quarters declining from 48·9 to 48·0 %.

Because only two of these thirty-seven cows were clinically affected in the period between the two measurements of the yields of the quarters, the results cannot be used to measure the effect of clinical mastitis on milk yield.

DISCUSSION

Our results confirm the findings of other workers (3)–(11) in that a relationship was found between the types of teat-cup liner used and the incidence of mastitis. No differences were found between the groups for new infection rate, teat orifice erosion or the leucocyte content of the milk. The herd was free of *Streptococcus agalactiae* infection, and about 62% of the cases of mastitis were caused by *Staphylococcus aureus*. These findings were thus much the same as in the Elveden herds (8).

Earlier experimental comparisons of the two liners used in this study (1) showed that cows milked with the extruded liner had a maximum milking rate about 10% higher and yields of machine strippings about 50% lower than when they were milked with the moulded liner.

Although the results from both milking and mastitis experiments show that the extruded liner used has a better performance, it is not possible to say whether or not the mastitis results are due to the difference in milking efficiency. There does not appear to be a good reason why a small increase in milking rate or a large decrease in the yield of strippings should affect the clinical incidence. As the Elveden data (8) were collected almost entirely during a period of hand stripping, it would not seem that inefficient stripping was the cause of the higher incidence of mastitis associated with the moulded liner. It is also difficult to understand why the use of the moulded liner should result in infected quarters becoming clinical while the leucocyte count of the milk from non-infected quarters was not influenced by the type of liner. It is still quite possible that, while not affecting a normal quarter (as judged by the leucocyte count) the action of a liner can irritate an infected quarter and so precipitate an attack of mastitis.

The main difference between the two types of liner is in the design of the mouthpiece, the barrels being similar except that the moulded liner has a larger internal diameter and is under a lower tension when in use. The mouthpiece of the moulded liner is less elastic and has a more rigid inner ring which makes a stronger vacuum seal with the teat and consequently 'holds' on to the teat more securely. Some of the earlier teat-cups had a metal hood to the liner that was said to cause obvious injury to the base of the teat. It is conceivable that the more rigid the mouthpiece the more friction there is on both the internal and external teat surfaces. Liners with relatively rigid, small mouthpieces are known to cause more teat congestion and this was a reason for the decision to change the type of liner used in two of the Elveden herds (8).

The effect of liner design on milk yield is inconclusive, and although the decline in milk yield with lactation was slightly greater for the quarters milked by moulded liners, it is not significant at the 5% level of significance. However, twenty-seven of the thirty-seven cows gave this response, so that more precise information is needed on this aspect of liner design.

These results should not be interpreted to mean that all extruded liners are better than moulded liners; only that the type of extruded liner used was superior to the moulded liner.

SUMMARY

1. An experiment was carried out to study the effect on udder health and milk yield when half-udders of dairy cows were milked with either a moulded or an extruded teat-cup liner.

2. There was a higher incidence of mastitis in quarters that were milked with moulded than with extruded liners, but the incidence of new infection was similar for each group of quarters.

3. The types of liners used had no measurable effect on teat orifice erosion or leucocyte content of the milk, nor was the decline in milk yield with advance in lactation significantly different for the groups of quarters.

We wish to thank Dr A. T. R. Mattick and Mr A. S. Foot for advice and encouragement, and Miss H. R. Chapman for assistance in the cleaning of teat-cup liners. We are indebted to Miss M. S. Hawkins and Messrs T. M. Higgs and D. S. Simpkin for technical assistance.

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650. THE INFLUENCE OF TEMPERATURE ON THE GENERATION TIME OF BACTERIA COMMONLY FOUND IN MILK

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INTRODUCTION

The generation rates of micro-organisms associated with milk-borne infections have been studied in various culture media, e.g. peptone, nutrient broth, glucose broth, under optimum temperature conditions. However, little attention has been paid to the use of milk as a culture medium for growth studies. Furthermore, it is not certain what effect temperatures other than the optimum have upon the generation rate of micro-organisms in this medium. Mason (1) has reviewed, and compiled from the literature, the generation times of various species of bacteria growing under optimal conditions.

The present study includes a determination of the generation times, in milk medium, of micro-organisms commonly associated with milk-borne infections at seven different temperatures, and may serve as a foundation for more exhaustive study by others.

EXPERIMENTAL

(1) *Standardization of inoculum*

For the preliminary standardization of inoculum two methods were employed: a direct method, and an indirect one.

The first method consisted in making serial dilutions in 9 ml. of nutrient broth, using 1 ml. of a 24 hr. culture of the test organism, and determining the number of viable cells by plating 0·1 ml. portions on blood agar. The extent of reduction in viable-cell concentration per tenfold dilution was thus determined, and reduction factors so produced were used as indices in approximating reductions in cell concentrations per tenfold dilution.

The second method involved the use of the Coleman universal spectrophotometer.

In determining the viable-cell concentration, serial dilutions were made in 9 ml. of nutrient broth, and the transmittance of each tube was determined before and after inoculation with the 24 hr. culture of the test organism. It was found that dilutions three to four places below the one which showed a detectable reduction in transmittance after inoculation would give from one to ten viable cells per 0·1 ml. portions upon plating on blood agar.

(2) *Methods and results*

Streptococcus haemolyticus (ATCC 9207), *Micrococcus pyogenes aureus* (ATCC P-209), *Escherichia coli*, strain B, *Bacillus subtilis*, variety *niger*, *Alcaligenes faecalis* (the above-mentioned three organisms were isolated from sewage), *Salmonella typhosa*, *Shigella dysenteriae* (from patients), and *Lactobacillus acidophilus* (isolated from milk) were chosen as representatives of eight genera commonly found in milk. All cultures were stored at 4° C., maintained by serial transfer on blood agar slants and in cooked meat medium

(Difco). Purity of cultures was determined by plating and by staining, and by biochemical tests.

Series of 5 × 50 mm. Pyrex test-tubes containing 1 ml. of milk sterilized at 15 lb. for 15 min., which had been brought up to the desired incubation temperature, were inoculated by means of 0·1 ml. pipettes with 0·1 ml. of the proper dilution of a 24 hr. culture of the test organism. As determined by previous experiments this inoculum contained one to ten living cells per 0·1 ml., the exact number being found by plating a similar portion on blood agar each time the inoculations were made. This quantity of inoculum prevents the exhaustion of nutrient materials, makes plate counts easy to read, and frees the experimenter from the necessity of making frequent dilutions. The cultures were immediately incubated at 4, room temperature (23–27), 30, 33, 37, 45 and 60° C. At exactly 3 hr., after making the proper allowance of time for the lag phase for each micro-organism (previously determined at each of the above seven temperatures), the cultures were removed from the incubators and immediately placed in the icebox. Then, one by one, the tubes were removed from the icebox, shaken well, and 0·1 ml. portions were plated on blood agar (BBL). Duplicate plates were made from each culture tube. Plates were inverted and incubated for 48 hr. at 37° C. before counting. *Micrococcus pyogenes aureus*, and *Streptococcus haemolyticus* were incubated in the presence of 10% carbon dioxide, since this induced higher plate counts. Plates giving 1–300 colonies were used for enumeration. The results obtained from these counts are shown in Tables 1–8.

Table 1. *Multiplication of* Lactobacillus acidophilus

Trial no.	Inoculum	Plate counts at 4° C.	Room	30° C.	33° C.	37° C.	45° C.	60° C.
1	6	5	30	50	70	350	330	0
2	4	4	20	30	40	200	190	0
3	3	2	15	20	35	150	140	0
4	5	5	25	40	60	260	230	0
	G.T.*	—	125·8	103·7	84·2	50·3	52·9	—

* Generation time in minutes.

Table 2. *Multiplication of* Bacillus subtilis

Trial no.	Inoculum	Plate counts at 4° C.	Room	30° C.	33° C.	37° C.	45° C.	60° C.
1	3	3	40	180	270	610	650	0
2	5	4	75	310	400	1020	1150	0
3	2	2	30	110	160	410	470	0
4	7	5	120	380	590	1660	1840	0
	G.T.*	—	45·4	30·7	28·3	23·2	22·7	—

* Generation time in minutes.

Table 3. *Multiplication of* Micrococcus pyogenes aureus

Trial no.	Inoculum	Plate counts at 4° C.	Room	30° C.	33° C.	37° C.	45° C.	60° C.
1	4	3	110	230	410	830	770	0
2	2	2	60	120	230	480	450	0
3	6	5	170	350	620	1310	1250	0
4	1	1	30	60	120	210	200	0
	G.T.*	—	38·2	30·4	26·6	23·2	23·5	—

* Generation time in minutes.

Table 4. *Multiplication of* Streptococcus haemolyticus

Trial no.	Inoculum	Plate counts at 4° C.	Room	30° C.	33° C.	37° C.	45° C.	60° C.
1	2	2	60	90	150	460	120	0
2	8	7	230	340	690	1750	450	0
3	5	5	160	240	410	1330	380	0
4	4	3	120	190	320	950	230	0
	G.T.*	—	37·1	32·7	27·4	22·5	30·9	—

* Generation time in minutes.

Table 5. *Multiplication of* Salmonella typhosa

Trial no.	Inoculum	Plate counts at 4° C.	Room	30° C.	33° C.	37° C.	45° C.	60° C
1	10	8	110	240	420	880	740	0
2	7	6	90	220	370	760	630	0
3	5	5	70	150	230	490	410	0
4	3	3	30	80	130	300	260	0
	G.T.*	—	50·3	38·1	32·7	27·1	28·2	—

* Generation time in minutes.

Table 6. *Multiplication of* Shigella dysenteriae

Trial no.	Inoculum	Plate counts at 4° C.	Room	30° C.	33° C.	37° C.	45° C.	60° C.
1	3	3	50	80	170	330	300	0
2	7	5	90	190	400	760	630	0
3	2	2	30	50	110	220	210	0
4	5	5	70	160	340	550	470	0
	G.T.*	—	47·2	37·6	31·5	26·1	26·6	—

* Generation time in minutes.

Table 7. *Multiplication of* Escherichia coli

Trial no.	Inoculum	Plate counts at 4° C.	Room	30° C.	33° C.	37° C.	45° C.	60° C.
1	4	4	80	340	1,060	6,300	5,900	0
2	3	2	60	290	1,050	4,300	4,100	0
3	6	6	120	570	2,000	10,800	10,400	0
4	5	4	110	500	1,750	8,100	7,900	0
	G.T.*	—	41·4	27·3	24·3	16·8	17·1	—

* Generation time in minutes.

Table 8. *Multiplication of* Alcaligenes faecalis

Trial no.	Inoculum	Plate counts at 4° C.	Room	30° C.	33° C.	37° C.	45° C.	60° C.
1	1	1	20	50	130	480	460	0
2	4	3	90	240	490	1640	1600	0
3	3	3	40	130	360	1350	1140	0
4	2	2	30	110	230	840	750	0
	G.T.*	—	42·9	32·0	26·1	23·2	23·6	—

* Generation time in minutes.

Temperatures, excepting room temperature, did not differ more than plus, or minus, 0·2° C. from those specified. Duplicate plates which differed by more than 20% were discarded. Conditions of timing, plating and counting were controlled so as to minimize the experimental errors. Homogenized milk obtained from a local dairy was used throughout this work. The same lots of media were employed for the entire study.

DISCUSSION

The procedure followed for determining generation time seemed satisfactory for ordinary practical purposes. Duplicates were found to be in close agreement, showing that the agitation of the milk tubes and the proper measurement of the inoculum had eliminated the sampling errors. Errors owing to unequal breaking up of the clumps formed in the process of multiplication are unavoidable. Obviously, these were small and not of sufficient magnitude to interfere with the interpretation of the results.

The results obtained are shown in Tables 1–8. The rate of reproduction is measured in terms of the generation time, which may be defined as the time in minutes necessary for the cells to double in number. Assuming that all are dividing, the generation time may be determined from the formulas given by Buchanan & Fulmer (2):

$$n = 3{\cdot}32\ (\log N - \log X_0),$$

$$\text{G.T.} = \frac{t_2 - t_0}{n},$$

in which n is the number of generations, N is the total population, X_0 is the number of cells in the inoculum, t_2 is the time at the end of the experiment, t_0 is the initial time, and G.T. is the generation time. The generation times of the various organisms studied were determined during the period of logarithmic growth. It was felt that accurate generation rates could only be obtained under optimum conditions for multiplication, and the logarithmic phase of growth is ideal for this purpose.

Milk held at 4° C. for 6 hr. showed no increase in the number of organisms initially present. This finding does not indicate that milk may be held indefinitely at this temperature. Pennington (3) found that milk stored at 0° C. underwent marked proteolysis which was noticeable at the end of 2 weeks. She stated that proteolysis of casein was due primarily to bacteria.

Temperatures ranging between 4 and 45° C. decreased the generation times of microorganisms, but while a consistent increase occurs in the rate of multiplication with increasing temperatures up to the optimum it is apparent that the effect of a given temperature increase near the optimum is less, compared to the effect produced by the same increase in the lower ranges.

The fast rates of reproduction of *Streptococcus haemolyticus* and *Micrococcus pyogenes aureus* at room temperature are worthy of note. Thus, if a sample of milk is allowed to stand at room temperature the first types of organisms developing in the milk will be streptococci and micrococci. These organisms find their way into milk from various sources, but chiefly from utensils, manure, feeds, and soil. Unless their action is inhibited by acid from lactobacilli and moulds, protein digestion in milk will result in a putrid odour and bitter flavour. This would occur most frequently in the fall and winter owing partly to the slower growth of other bacteria in cold weather. Compared with *Strepto-*

coccus haemolyticus and *Micrococcus pyogenes aureus*, *Escherichia coli* seems to have a longer generation time at room temperature. However, at its optimum temperature *E. coli* is the most rapidly reproducing organism of all those commonly found in milk. The observed generation time of *E. coli* of 16·8 min. at 37° C. is longer than that of 12·5 min. reported by Frazier (4).

Alcaligenes faecalis grows at about the same rate as *Escherichia coli*. This organism is undesirable in milk because it may cause coagulation of casein. The alkaline reaction produced by this organism is caused principally by the fermentation of citric acid salts and other salts of organic acids to alkali carbonates which split fat and cause rancidity in milk.

The cultures of *Lactobacillus acidophilus* investigated were by far the most slowly growing at all temperatures; at 37° C. they gave a generation time of 50·3 min. According to England (5) the generation time of *L. acidophilus* in milk is 67–87 min. at 37° C.

Salmonella typhosa, the causative agent of typhoid fever, and *Shigella dysenteriae*, the causative agent of diarrhoea, have slow generation rates at room temperature. This is, of course, fortunate, for their activity will be inhibited by streptococci, micrococci, and other bacteria which outgrow them at this temperature. At their optimum temperature the generation rates of these micro-organisms compare fairly closely with the multiplication rate of *Bacillus subtilis*. *B. subtilis* is responsible for the coagulation of casein by the enzyme rennin secreted by this organism.

SUMMARY

An attempt to show the effect of temperature on the rate of growth of bacteria in milk was made by finding the generation times of *Salmonella typhosa*, *Shigella dysenteriae*, *Streptococcus haemolyticus*, *Micrococcus pyogenes aureus*, *Lactobacillus acidophilus*, *Escherichia coli*, *Bacillus subtilis* and *Alcaligenes faecalis* at temperatures ranging from 4 to 60° C. Growth was quantitatively measured by means of plate counts during the logarithmic period which was previously determined for each organism. The following is a summary of the results obtained:

At 4° C. none of the micro-organisms showed evidence of multiplication during the 6 hr. incubation.

As temperatures of 5–45° C. were approached the generation time decreased until the optimum temperature for each organism was reached; beyond this point a slowing of growth was observed, until at 60° C. viability was apparently lost by all the organisms.

Streptococcus haemolyticus and *Micrococcus pyogenes aureus* have a generation time of 37–23 min.

Salmonella typhosa and *Shigella dysenteriae* have a generation time of 50–26 min.

Escherichia coli and *Alcaligenes faecalis* have a generation time of 41–16 min.

Bacillus subtilis grows at about the same rate as *Alcaligenes faecalis*.

The slowest organism of all appears to be *Lactobacillus acidophilus* with a generation time of 52–125 min.

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651. THE LACTOSE-CHLORIDE CONTRIBUTIONS TO THE FREEZING-POINT DEPRESSION OF MILK

II. EXAMINATION OF PARTIAL CONTRIBUTIONS OVER THE FULL LACTATION PERIOD OF TWO COWS

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(With 3 Figures)

Much evidence has been accumulated to show that constancy of freezing-point depression is one of the most characteristic properties of milk, particularly in bulked samples (1)–(7). However, most work has had as its aim the use of the constant for the detection of added water, and less information is available regarding the partial contributions of different constituents and the inter-relationships of these partial contributions. Surveys of a general nature have been carried out by Aschaffenburg & Temple (8), who did not find a close correlation between solids-not-fat and freezing-point depression over a full lactation period, and by Aschaffenburg & Veinoglou (9), who reported normal freezing-point depressions with low solids-not-fat content. Scott-Dodd & Cowan (10) and Rowland, Aschaffenburg & Veinoglou (11) have reported similarly on samples from cows infected with mastitis.

The lactose and chloride contents have been studied and it is known that each may vary considerably (12)–(20). Previous statistical analyses have indicated that together they contribute about 70% of the total freezing-point depression. Since the total depression remains fairly constant, the conclusion is that the lactose and chloride are related in some complementary manner. Statements appear that a lower content of lactose is counter-balanced by a rise in chloride content, and formulae have been deduced from which it is claimed that the approximate lactose content may be calculated from the chloride content (21). Barry & Rowland (22), in examining the relationship between ionic constituents and lactose, found that a linear relationship existed between the sodium, potassium and chloride, and the lactose. In particular, it was found that with a fall in lactose there occurred a linearly related rise in chloride content. From their graphs a change of 0·1% chloride ion appears to be equivalent to 1·75% lactose (monohydrate).

It was shown previously (23), that the contributions of lactose and chloride to the total freezing-point depression were directly proportional to concentration. Incremental additions to watered milk indicated that the depression due to 5% lactose (monohydrate) was 0·294° C., and that due to 0·1% chloride (as chloride ion), was 0·110° C. From this a change of 0·1% chloride may be regarded as equivalent to 1·87% lactose. For most purposes the two equivalents show reasonable agreement.

With respect to these constituents two problems may be considered to arise. The first is whether the sum of the partial depressions remains a constant fraction of the total

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depressions and consequently, whether the lactose and chloride contributions maintain precisely the inverse relationship discussed above, over a lactation period.

Using the straight-line relationship between concentrations of lactose and chloride and the partial freezing-point depression, it is possible to calculate the fractions of the freezing-point depression that are due to each from the determined concentrations of these two constituents. An approach of this kind is desirable since other comparisons have been deduced only from the percentage contents of lactose and chloride. It may be noted that factors other than lactose and chloride should be considered with respect to freezing-point changes. This point was studied by Davies(19), who concluded that an isotonic diluent was present in milks of low solids-not-fat content.

The aim of the present work has been: (1) to test the application of conversion factors relating concentration of lactose and chloride to partial freezing-point depressions over a full lactation period; (2) to examine the proportion of the total depression represented by the sum of the partial contributions so calculated; and (3) to examine the inter-relationships of the lactose and chloride partial depressions over the period.

EXPERIMENTAL

Weekly samples of morning milk from one Jersey and one Friesian cow of the stud herds of the Hawkesbury Agricultural College were collected and forwarded immediately for analysis. Adequate precautions were taken to keep the milk in good condition, and methylene blue reductase tests were made on each sample. The result was never less than 9 hr. after collection. The first samples were taken 5 and 4 weeks after calving for the Jersey and Friesian respectively.

Freezing-point depressions in triplicate were measured in a Hortvet cryoscope. Total solids, fat and protein (total N × 6·38) were determined in duplicate by standard methods. Lactose was determined in duplicate, as previously(23), according to the *Methods of the Association of Official Agricultural Chemists*, 7th ed., p. 232. Chloride determinations were made in duplicate by the method of Davies(16). Results are given in Tables 1 and 2.

TREATMENT OF RESULTS

Total freezing-point depression. While the constancy of the depression has been fairly thoroughly investigated, it is of interest to consider the results shown in Fig. 1. With the Jersey cow, the minimum depression was 0·531° C., the maximum 0·550° C., and the mean 0·542° C. with a coefficient of variation of 4·9%. The Friesian showed similar variations about slightly greater depressions. The minimum was 0·540° C., the maximum 0·562° C., and the mean 0·550° C., with a coefficient of variation of 5·5%. Examination of Fig. 1 shows that changes in the freezing-point depression for the milk from one cow were generally associated with similar changes in the depression for the milk of the other, although such changes were not invariably in step. However, one implication is that in general both cows may have responded to climatic and other conditions in a similar way.

It is of further interest to consider these changes, since it is clear that although the Friesian had a consistently lower chloride level, only on three occasions was the freezing-point depression less than that of the Jersey, and then only to a very slight extent (0·002° C.).

Table 1. *Analytical results of milk from Jersey cow 'Richmond Lantana'*

Weekly sample no.	Total solids (%)	Fat (%)	Solids-not-fat (%)	Lactose (as monohydrate) (%)	Chloride (mg. Cl^-/100 g.)	Protein (%)	Freezing-point depression (° C.)
1	13·08	4·40	8·68	4·41	117	3·57	0·545
2	13·36	4·45	8·89	4·18	127	4·01	0·548
3	14·20	5·15	9·03	4·56	110	3·77	0·546
4	15·97	7·25	8·72	4·36	118	3·66	0·544
5	15·49	6·70	8·82	4·66	116	3·46	0·538
6	14·99	5·90	8·99	4·92	96	3·37	0·548
7	14·75	6·15	8·60	5·13	120	2·77	0·538
8	14·90	5·95	8·95	4·70	113	3·55	0·542
9	14·44	5·05	9·39	4·80	106	3·89	0·550
10	15·90	6·70	9·20	4·74	105	3·76	0·550
11	15·49	6·35	9·14	4·68	108	3·76	0·543
12	15·00	5·65	9·35	4·63	102	4·02	0·545
13	14·27	4·90	9·37	4·86	103	3·81	0·544
14	15·71	6·80	8·91	4·70	98	3·51	0·533
15	14·21	5·20	9·01	4·65	120	3·66	0·539
16	14·01	4·65	9·36	4·86	101	3·80	0·536
17	14·02	4·85	9·07	4·77	103	3·60	0·538
18	15·14	5·70	9·44	4·84	95	3·90	0·548
19	14·14	4·75	9·39	4·96	104	3·73	0·538
20	14·93	5·80	9·13	4·68	98	3·75	0·539
21	14·89	5·35	9·54	4·86	115	3·98	0·545
22	14·73	5·30	9·43	4·66	121	4·07	0·546
23	15·44	6·20	9·24	4·65	101	3·89	0·539
24	14·54	5·35	9·17	4·52	124	3·95	0·549
25	14·22	5·10	9·12	4·60	103	3·82	0·531
26	14·28	5·00	9·28	4·68	113	3·90	0·541
27	14·13	4·90	9·23	4·52	123	4·01	0·542
28	14·90	5·80	9·10	4·69	115	3·71	0·541
29	15·78	6·95	8·83	4·70	110	3·43	0·543
30	14·05	4·90	9·15	4·76	110	3·69	0·540

Table 2. *Analytical results of milk from Friesian cow 'Hawkesbury Rosemaid'*

Weekly sample no.	Total solids (%)	Fat (%)	Solids-not-fat (%)	Lactose (as monohydrate) (%)	Chloride (mg. Cl^-/100 g.)	Protein (%)	Freezing-point depression (° C.)
1	12·10	3·35	8·74	4·81	76	3·23	0·561
2	11·58	3·15	8·42	4·55	87	3·17	0·555
3	11·74	3·45	8·27	4·68	100	2·89	0·554
4	11·64	3·05	8·59	4·96	84	2·93	0·554
5	11·93	3·40	8·54	4·82	92	3·02	0·548
6	11·89	3·40	8·49	4·75	86	3·04	0·551
7	12·10	3·20	8·90	4·65	87	3·55	0·548
8	12·16	3·80	8·36	4·85	85	2·81	0·544
9	12·48	3·65	8·83	4·90	88	3·23	0·554
10	13·76	5·10	8·66	4·71	88	3·25	0·548
11	12·68	3·80	8·88	4·64	100	3·54	0·549
12	12·20	3·55	8·65	4·89	96	3·06	0·542
13	12·17	3·45	8·72	4·81	95	3·21	0·552
14	12·31	3·70	8·61	4·83	94	3·08	0·545
15	11·84	3·25	8·59	4·79	93	3·10	0·548
16	11·76	3·10	8·66	4·88	84	3·08	0·546
17	12·40	3·65	8·75	4·80	78	3·25	0·540
18	13·15	4·15	9·00	4·98	80	3·32	0·557
19	12·43	3·60	8·83	4·91	87	3·22	0·548
20	12·16	3·65	8·51	4·87	83	2·94	0·549
21	12·62	3·90	8·72	4·73	97	3·29	0·554
22	12·43	3·90	8·53	4·48	112	3·35	0·542
23	12·59	4·00	8·59	4·66	101	3·23	0·545
24	12·52	3·85	8·67	4·72	106	3·25	0·557
25	12·70	4·10	8·60	4·80	96	3·10	0·546
26	12·90	3·90	9·00	4·79	100	3·51	0·554
27	13·56	4·40	9·16	4·64	111	3·82	0·562
28	13·49	4·40	9·09	4·74	95	3·65	0·545
29	14·14	4·95	9·19	4·69	96	3·80	0·546
30	13·30	3·90	9·40	4·95	96	3·75	0·557

Lactose and chloride contribution to total freezing-point depression

Since the conversion factors for lactose and chloride relating concentration to partial freezing-point were determined previously (23) in the presence of practically constant fat and protein concentrations, they may be regarded as relating essentially to milk on a fat and protein-free basis. For this reason results, corrected for fat and protein content, are presented in Tables 3 and 4, together with the calculated partial depressions and their

Fig. 1. Total freezing-point depressions for thirty weekly samples of milk from one Friesian and one Jersey cow.

Table 3. *The lactose and chloride contents of the milk of the Jersey cow and the partial freezing-point depressions*

Sample no.	Lactose* monohydrate (%)	Partial depression due to lactose (° C.)	Chloride (as Cl^-)* (mg./100 g.)	Partial depression due to chloride (° C.)	Sum of partial depressions (° C.)	Total depression (° C.)	Sum of partial depressions as % of total
1	4·78	0·282	127	0·140	0·422	0·545	77·5
2	4·57	0·275	139	0·153	0·428	0·548	78·1
3	5·02	0·295	120	0·132	0·427	0·546	78·2
4	4·89	0·291	132	0·145	0·436	0·544	80·2
5	5·19	0·306	129	0·142	0·448	0·538	83·2
6	5·38	0·317	106	0·117	0·434	0·548	79·2
7	5·61	0·332	132	0·148	0·480	0·538	89·2†
8	5·18	0·305	125	0·137	0·442	0·542	81·4
9	5·28	0·311	117	0·129	0·440	0·550	80·1
10	5·29	0·312	118	0·130	0·442	0·550	80·3
11	5·23	0·308	130	0·143	0·451	0·543	83·1
12	5·13	0·302	113	0·124	0·426	0·545	78·2
13	5·33	0·313	113	0·124	0·437	0·544	80·5
14	5·24	0·308	109	0·120	0·428	0·533	80·4
15	5·11	0·301	131	0·144	0·445	0·539	82·6
16	5·32	0·313	110	0·121	0·434	0·536	80·9
17	5·22	0·307	112	0·123	0·430	0·538	79·8
18	5·36	0·316	105	0·116	0·432	0·548	78·7
19	5·43	0·320	113	0·124	0·444	0·538	82·4
20	5·17	0·304	108	0·119	0·423	0·539	78·6
21	5·36	0·316	126	0·139	0·455	0·545	83·4
22	5·14	0·303	134	0·147	0·450	0·546	82·3
23	5·17	0·304	113	0·124	0·428	0·539	79·5
24	4·98	0·293	137	0·151	0·444	0·549	80·9
25	5·04	0·296	114	0·124	0·421	0·531	79·3
26	5·14	0·303	124	0·136	0·439	0·541	81·1
27	4·94	0·291	134	0·147	0·438	0·542	80·9
28	5·18	0·305	127	0·140	0·445	0·541	82·2
29	5·23	0·308	123	0·135	0·443	0·543	81·6
30	5·18	0·305	121	0·133	0·438	0·540	81·2

* On fat and protein-free basis. † Not included in analysis of results.

sum. The contributions of the lactose and chloride are presented graphically in Figs. 2 and 3, where the inverse relationship of lactose and chloride and the upward trend towards the end of the period for the Friesian may be better appreciated. At first sight the sum of the contribution appears to be rather constant at about 80% for the Jersey, and 75% for the Friesian. Closer examination, however, was made as follows.

Table 4. *The lactose and chloride contents of the milk of the Friesian cow and the partial freezing-point depressions*

Sample no.	Lactose* monohydrate (%)	Partial depression due to lactose (° C.)	Chloride (as Cl^-)* (mg./100 g.)	Partial depression due to chloride (° C.)	Sum of partial depressions (° C.)	Total depression (° C.)	Sum of partial depressions as % of total
1	5·16	0·304	82	0·090	0·394	0·561	70·2
2	4·87	0·287	93	0·105	0·392	0·555	70·6
3	4·98	0·293	107	0·118	0·411	0·554	74·3
4	5·28	0·311	90	0·099	0·410	0·554	74·1
5	5·15	0·303	98	0·108	0·411	0·548	74·9
6	5·08	0·299	92	0·101	0·400	0·551	72·7
7	5·00	0·294	93	0·102	0·396	0·548	72·3
8	5·18	0·305	91	0·100	0·405	0·544	74·6
9	5·26	0·309	94	0·103	0·412	0·554	74·4
10	5·14	0·302	96	0·106	0·408	0·548	74·5
11	5·02	0·295	108	0·119	0·414	0·549	75·4
12	5·24	0·308	102	0·112	0·420	0·542	77·4
13	5·16	0·304	101	0·111	0·415	0·552	75·3
14	5·18	0·305	100	0·110	0·415	0·545	76·2
15	5·12	0·301	99	0·109	0·410	0·548	74·8
16	5·22	0·307	90	0·099	0·406	0·546	74·4
17	5·16	0·304	84	0·092	0·396	0·540	73·3
18	5·39	0·317	87	0·096	0·413	0·557	74·2
19	5·27	0·310	93	0·102	0·412	0·548	75·2
20	5·23	0·307	89	0·098	0·405	0·549	73·9
21	5·09	0·300	103	0·113	0·413	0·554	74·4
22	4·84	0·284	119	0·131	0·415	0·542	76·4
23	5·03	0·293	108	0·119	0·415	0·545	76·2
24	5·08	0·299	113	0·124	0·423	0·557	75·8
25	5·17	0·305	102	0·112	0·417	0·546	76·4
26	5·17	0·305	107	0·118	0·423	0·554	76·4
27	5·06	0·298	121	0·133	0·431	0·562	76·6
28	5·16	0·304	103	0·113	0·417	0·545	76·6
29	5·13	0·301	105	0·116	0·417	0·546	76·4
30	5·36	0·315	104	0·115	0·430	0·557	77·2

* On fat and protein-free basis.

Analysis of results

Labelling the total freezing-point depression $x°$ C., and the depression due to the lactose-chloride content on a fat and protein-free basis $Y°$ C., it was anticipated that

$$Y = \beta x + U, \tag{1}$$

where β is a constant, and U a random variable with zero mean, i.e., apart from random fluctuation, the proportion of the total depression accounted for by lactose and chloride was expected to be constant (and equal to β, with 100β about 75–80%).

In more detail, the x values were taken to be relatively error free compared with the Y values (especially as they were the means of three determinations). Further, the Y values about their expected values βx were supposed to be normally distributed with constant variance σ^2, to a reasonable degree of approximation. (This certainly ignores any suggestion of seasonal trend, mentioned above.) In these circumstances analyses

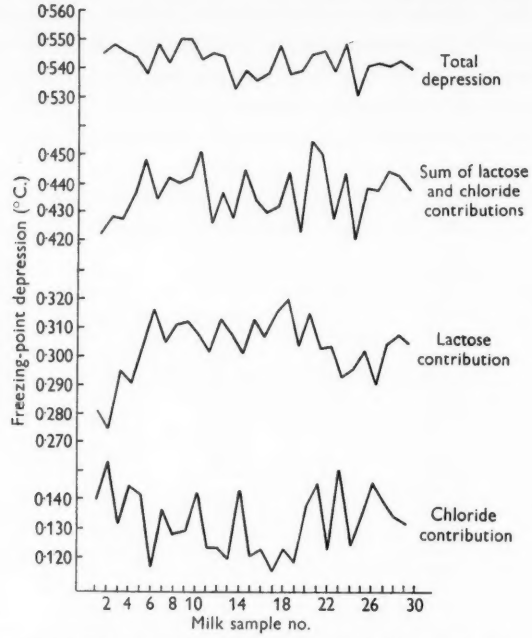

Fig. 2. The contribution of lactose and chloride to the total freezing-point depression for the Jersey milk.

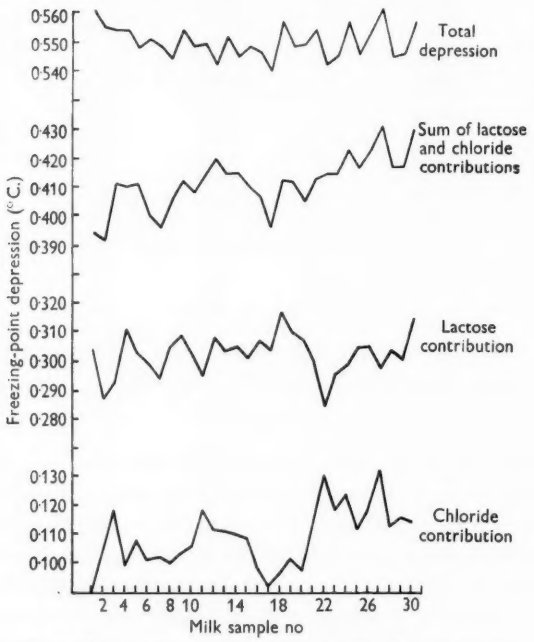

Fig. 3. The contributions of lactose and chloride to the total freezing-point depression for the Friesian milk.

were made to determine, first, whether the data could be satisfactorily represented by a relation such as that in equation (1), i.e., whether the anticipation of a constant proportion of total depression due to lactose and chloride could be justified, this analysis being possible because of the occurrence of several Y values for the same x value; and secondly, in the event of a satisfactory representation by (1), the best estimate of β (see Appendix I).

For the Jersey, the hypothesis of proportionality was in agreement with the data, and the appropriate estimate of β over the whole period was 0·805—i.e. about 80% of the depression could be ascribed to lactose-chloride. But for the Friesian, the hypothesis of proportionality could not be maintained, and consequently no valid estimate of β could be given. Ignoring this failure of the hypothesis, about 75% of the total depression could be ascribed to lactose-chloride.

Inter-relation of partial depressions of lactose and chloride

As indicated above, freezing-point depressions of the Friesian samples were greater than those for the Jersey samples, despite the consistently lower chloride content of the Friesian milk. Lactose variations between the milks of the two cows were comparatively slight. It would appear then that the lower proportion of the total freezing-point depressions of the Friesian milk for the sum of the lactose and chloride contributions was due in the main to the lower chloride contribution. It appears also that other osmotic factors must have been operating sufficiently to counterbalance the lower chloride level, and to cause a greater total freezing-point depression. This follows from the fact that the results reported confirm that despite other changes the lactose and chloride components were complementary.

Supposing a linear relation between the components (cf. Davies[21] and Barry & Rowland[22]), and working in partial depressions in ° C. with chloride regarded as the independent variable, it was found that:

$$\text{for the Jersey} \quad L = 0{\cdot}382 - 0{\cdot}589C,$$
$$\text{for the Friesian} \quad L = 0{\cdot}339 - 0{\cdot}339C,$$

where L and C are the partial depressions in ° C. of the lactose and chloride respectively. Tests of the adequacy of the supposition of linearity were applied (see Appendix II for details), and linearity accepted for both animals.

Thus the lactose and chloride contributions were complementary, but perhaps at different levels—an investigation of this point (Appendix II) indicated that the difference between the coefficients −0·589 for the Jersey and −0·339 for the Friesian was not statistically significant, and the joint regression was found to be

$$L = 0{\cdot}359 - 0{\cdot}466C$$

in the above notation. Whether any difference that may exist is a breed effect or merely a reflexion of the behaviour of two individual cows is a question which might well form the subject of survey work.

It may be that the proportionality hypothesis with respect to the gross freezing-point depression is in fact too great a simplification of the situation and is to be regarded as an approximation only. This was so with the Friesian, where there was a departure from this concept, although the lactose and chloride remained complementary. Although the differences between the constants in these complementary relationships for the Jersey and

Friesian milks were not statistically significant, they were certainly considerable and may well be biologically important. The fact that they were so considerable casts doubt on the validity of formulae for calculating the concentration of either lactose or chloride from that of the other, particularly in milk from individual cows as distinct from samples of bulked milk.

SUMMARY AND CONCLUSIONS

1. In testing the technique of using conversion factors (from determined concentration) for the calculation of the partial freezing-point depressions of lactose and chloride, regular weekly chemical analyses and freezing-point determinations have been made on the milk of one Jersey and one Friesian cow over a period of 7 months.

2. Relative constancy of freezing-point has again been noted, but slight though consistent differences were present between the milks of the two cows. No seasonal variations were observed, but changes in the freezing-point depression for the milk of one were generally associated with corresponding changes for the milk of the other.

3. Linear relationships were found and their adequacy tested between the partial depressions due to lactose and chloride for each animal. The relationships, though not statistically significantly different, differed considerably for each animal. It is suggested that formulae for the calculation of one of the constituents, lactose or chloride, from the determined amount of the other, need to be treated with caution in view of the results obtained for the two animals in this work.

For the Jersey milk it was found that 80% of the total freezing-point depression was due to the lactose and chloride content (on a fat and protein-free basis). For the Friesian milk, indications were that the lactose-chloride contribution was of the order of 75% of the total depression, but the result of statistical analysis was less satisfactory for this milk.

The co-operation of Mr J. McGibbon of the Hawkesbury Agricultural College is gratefully acknowledged. Thanks are due also to Mr J. V. Pascoe and the Dairy Farmers Co-operative Milk Co. Ltd. for their continued interest in the work.

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APPENDIX I

The numerical analyses were carried out with the depressions expressed in $\frac{1}{1000}$° C. as unit. Estimates of β, written b, were determined from

$$b=\frac{\Sigma x_i Y_i}{\Sigma x_i^2},$$

with x_i and Y_i corresponding depressions (see text for definitions) and the estimates of σ^2, written S^2, from

$$S^2=\frac{\Sigma (Y_i-bx_i)^2}{N-1}=\frac{\Sigma Y_i^2-(\Sigma x_i Y_i)^2/\Sigma x_i^2}{N-1},$$

with N pairs of observations available.

Jersey

(i) Investigation of proportionality.

Source	S.S.	D.F.	Mean square	Ratio
Slope	5,535,501·34	1	5,535,501·34	65990****
About regression	2,348·66	28	83·88	—
Means about regression	911·66	14	65·12	0·633
Within groups about means	1,437·00	14	102·64	—
Total (Y)	5,537,850·00	29	—	—

(ii) $b=0{\cdot}805$, with the central 95% confidence interval for β of (0·799, 0·812), $S^2=83{\cdot}9$.

Friesian

(i) Investigation of proportionality.

Source	S.S.	D.F.	Mean square	Ratio
Slope	5,080,665·15	1	5,080,665·15	53250****
About regression	2,766·85	29	95·41	—
Means about regression	2,202·52	13	169·42	4·80**
Within groups about means	564·33	16	35·27	—
Total (Y)	5,083,432·00	30	—	—

(ii) $b=0{\cdot}748$, $S^2=95{\cdot}4$.

APPENDIX II

Writing Y for the partial depression in $\frac{1}{1000}$° C. due to lactose and x for that due to chloride, and supposing $Y=\alpha+\beta x+U$, where α and β are constants with U a normal variate with zero mean and variance σ^2, led to the analyses of variance and estimates given below.

Jersey

(i) Investigation of linearity.

Source	S.S.	D.F.	Mean square	Ratio
Slope	1149·5	1	1149·5	16·8***
About regression	1845·2	27	68·3	—
Means about regression	1148·7	21	54·7	0·47
Within groups about means	696·5	6	116·1	—
Total (Y)	2994·8	28	—	—

(ii) Estimated relation: $Y=382-0{\cdot}589x$, with σ^2 estimated as 68·3.

Friesian

(i) Investigation of linearity.

Source	S.S.	D.F.	Mean square	Ratio
Slope	371·18	1	371·18	8·54**
About regression	1217·12	28	43·47	—
Means about regression	994·62	22	45·21	1·22
Within groups about means	222·50	6	37·08	—
Total (Y)	1588·30	29	—	—

(ii) Estimated relation: $Y=339-0{\cdot}339x$, with σ^2 estimated as 43·5.

Joint relation

(i) Investigation of adequacy of joint relation.

Source	S.S.	D.F.	Mean square	Ratio
Joint regression	1418·18	1	1418·18	25·4***
Between regressions	102·53	1	102·53	1·84
Sum of separate regressions	1520·71	2	—	—
About separate regressions	3062·35	55	55·68	—
Total	4583·06	57	—	—

(ii) Estimated relation: $Y=359-0{\cdot}466x$, with σ^2 estimated as 55·7.

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652. MICROSCOPIC APPEARANCE OF FAT ON THE MILK SURFACE AS AFFECTED BY APPLICATION OF SURFACE-ACTIVE SUBSTANCES TO THE SURFACE

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While the behaviour of the fat globule membrane is of paramount importance in so many phenomena in milk (clumping, churning, homogenization, etc.), there are unfortunately few techniques available for study of its physico-chemical properties. The use of surface microscopy to observe the behaviour of fat globules on the surface of milk gives one valuable if somewhat indirect approach to this subject, since the penetration of fat globules into the surface reflects a change to a hydrophobic condition of the globule membrane. Surface-active substances (alcohols and synthetic surfactants) are used to alter the properties of the fat globule membrane in several methods of fat determination (cf. King(5)) and to release milk fat for chemical study (Patton(9)). They have also been used to improve the dispersing properties of whole milk powders (Hibbs & Ashworth(1); Mather & Hollender(7); Hollender(2)). Study of the effect of surface-active substances on the behaviour of fat on the surface of milk should bear directly on these methods, as well as reveal something of the physico-chemical behaviour of the fat globule membrane.

In one of the earlier investigations on the microscopic structure of the surface of milk the author(4) observed that certain surface-active substances when applied to the surface brought a large number of fat globules and clumps (at higher temperatures, also lenses and patches) to the surface. These substances presumably reach the fat globules close to the milk surface, and interact with the globule membrane in such a way that its outer layer is rendered water-repellent. Even on the surface of skim milk, which usually contains only a few fat globules, there appeared large areas of numerous small dark fat globules and bright patches. With some other surface-active substances, however, the milk surface was swept clean of microscopically visible fat forms by the spreading out of a very thin film of this substance. To the first group belonged *iso*-butyl, *iso*-amyl and benzyl alcohols and monobutyrin; to the second group oxidized butterfat, oleic acid, triolein, sodium oleate and tributyrin. The non-surface-active petroleum ether did not exhibit any of these phenomena.

On the other hand, the milk surface will also be enriched with fat globules, clumps, lenses and patches by mechanical disturbance of this surface, either by repeated touching with a platinum ring or by allowing air bubbles to burst there (King(6)).

The present paper reports observations on the effect of a number of other surface-active substances on the fat on the surface of milk.

EXPERIMENTAL METHOD

The microscopic equipment was the same as described in the previous paper(6)—a simple prism vertical illuminator in conjunction with a short mounted objective $\times 10$, binocular tube $\times 1{\cdot}5$ and the eyepieces $\times 10$. The fat globules and clumps on the milk surface

were counted by means of a calibrated net eyepiece micrometer, usually in ten view fields.

For the tests with the substances which cause rising of the fat globules to the milk surface the overflow outfit described in the previous paper was used (6). The diameter of the inner bowl was 20 mm. The milk surface was cleaned by overflowing 10–15 ml. of milk, after which the surface was almost free of microscopically visible fat forms. The substance was applied by touching the milk surface in the centre with a platinum loop of inner diameter 1·9 mm. containing about 0·0005–0·001 g. of the substance.

The tests with substances which sweep the surface clean were made on microscope slides with cavities containing a large drop of milk (about 1·5 ml.) by applying the platinum loop containing the substance to the centre of the surface. Here the surface was covered at the beginning with a moderate number of fat globules and small clumps (about 500–700 per sq.mm.) which allowed the spreading phenomenon to be followed.

The spreading-out of the surface-active substance in both cases took place very quickly.

The milk used was fresh, unpasteurized, with a fat content of about 4%.

RESULTS

A. *Surface-active substances bringing the fat globules on the milk surface*

To study the influence of the chain length of the hydrophobic hydrocarbon group of the alcohol on its behaviour on the milk surface, the first ten normal members of the homologous series of monohydric aliphatic alcohols were tested. The observations were carried out at temperatures of 16–23° C.

The qualitative picture was as follows:

Methyl and ethyl alcohols. Moderate number of single dark fat globules on milk surface.

n-*Propyl and* n-*butyl alcohols.* Large number of single dark fat globules and some few clumps on milk surface.

n-*Amyl alcohol.* Large number of single dark fat globules; quite a number of clumps of different sizes.

n-*Hexyl and* n-*heptyl alcohols.* Large number of single dark globules; quite a number of middle-sized (diam. 20–40 μ) and large (diam. 75–150 μ) clumps (apparently patches of alcohol crowded with dark fat globules).

n-*Octyl and* n-*nonyl alcohols.* Large lenses of alcohol interspersed in a pattern of crowded single dark globules; around the lenses more or less empty zones. In 3–5 min. the dark globules begin to concentrate in these lenses, and after about 15 min. the lenses have been converted into large (diam. 75–150 μ) aggregations of dark globules, of irregular shape.

n-*Decylalcohol.* Large lenses of alcohol shooting showers of dark fat globules into a pattern of crowded globules; around the lenses empty zones. In some few minutes the lenses take an irregular shape and, beginning from the periphery, a concentration of dark globules in the lenses takes place. At last the lenses crowded with fat globules are subdivided into smaller aggregations of dark globules. These aggregations are interspersed in a pattern of crowded single globules.

The surface distribution of the single fat globules was uniform except with methyl alcohol. This also applies to the 'globule areas' which contained interspersed aggregations of globules (beginning with *n*-amyl alcohol).

The diameter of the spherical segments of globules ranging above the surface varied between 1 and 8 μ.

With the alcohols of lower solubility the excess of alcohol appears first on the milk surface as large lenses, which in a short time take up large numbers of fat globules and then disintegrate into clumps of irregular size.

Table 1. *The effect of the chain length of the monohydric aliphatic alcohols on the number of fat globules brought on to the milk surface*

Milk sample		A (6. xii. 55)	B (7. xii. 55)	C (4. i. 56)	D (5. i. 56)	E (19. i. 56)	F (20. i. 56)	
Measuring temperature (° C.)		19–20	20–23	17	16–17	19	20	
	Water solubility (g./100 ml.)	No. of fat globules per sq.mm. of the milk surface						
Methyl alcohol	∞	360	—	500	—	210	—	
Ethyl alcohol	∞	580	—	920	—	3,820	—	
n-Propyl alcohol	∞	5,400	—	10,200	—	10,210	—	
n-Butyl alcohol	7·9	6,660	—	8,820	—	12,560	—	
n-Amyl alcohol	2·7	5,400	4,500	11,550	—	7,850	—	No. of globules in 'globule areas'
n-Hexyl alcohol	0·6	—	4,860	8,530	—	7,600	—	
n-Heptyl alcohol	0·1	—	6,300	—	10,880	9,740	—	
n-Octyl alcohol	0·06	—	6,300	—	10,330	9,200	—	
n-Nonyl alcohol	—	—	5,760	—	10,290	—	10,960	
n-Decyl alcohol	—	—	5,760	—	9,280	—	—	

Table 1 shows the effect of the chain length of the monohydric aliphatic alcohols on the amount of the fat globules brought to the surface of unpasteurized whole milk (at temperatures between 16 and 23° C.). There is a steep increase in the number of fat globules penetrating the milk surface with increasing chain length beginning with *n*-propyl alcohol. This rise is also shown in Tables 4 and 5. It is of interest that the phenomenon is independent of the solubility, since *n*-propyl alcohol as well as methyl and ethyl alcohols are completely miscible with water. While the water solubility of the alcohols rapidly decreases with increasing chain length—starting with *n*-butyl alcohol—the number of fat globules brought to the surface does not show any definite relationship to the chain length, but remains roughly on the level attained by *n*-propyl alcohol.

Table 2. *The effect of isomeric alcohols on the number of fat globules brought on to the milk surface*

Milk sample		G (14. xii. 55)	H (6. i. 56)
Measuring temperature (° C.) ...		22	19
	Water solubility (g./100 ml.)	No. of fat globules per sq.mm. of milk surface	
prim.-Butyl alcohol	7·9	5,330	9,620
sec.-Butyl alcohol	12·5	7,560	10,630
tert.-Butyl alcohol	∞	6,840	9,490

According to Table 2 there is almost no difference in the behaviour of three isomeric alcohols, *n*-primary, secondary and tertiary butyl alcohols, and no relationship with the solubility figures.

Table 3. *The effect of double bond and phenyl group on the number of fat globules brought on to the milk surface*

Milk sample		G (14. xii. 55)	H (6. i. 56)
Measuring temperature (° C.) ...		21–22	17–18
	Water solubility (g./100 ml.)	No. of fat globules per sq.mm. of milk surface	
Allyl alcohol	∞	5,220	1,680
Benzyl alcohol	4	1,580	1,810
Phenyl ethyl alcohol	—	4,460	3,910

Table 3 gives data on allyl alcohol, containing a double bond, and on benzyl and phenylethyl alcohols, containing the phenyl group. With the last two there appear lenses of alcohol on the milk surface, surrounded by a narrow empty zone. However, unlike the lenses of hexyl to decyl alcohols, they do not concentrate the fat globules in their anterior.

Table 4. *The effect of ageing on the number of fat globules brought on to the milk surface. Milk aged* 19 *hr. at* 3–4° *C.*

Milk sample	I (26. i. 56)	
	Fresh	Aged
Measuring temperature (° C.)	21	21
	No. of fat globules and clumps per sq.mm. of milk surface	
Ethyl alcohol	1,260 globules	1,640 globules and clumps
n-Propyl alcohol	8,950 globules	10,160 globules
n-Butyl alcohol	9,830 globules	11,680 globules and clumps
n-Amyl alcohol	5,920 globules and clumps	7,390 globules and clumps

As seen from Table 4 there occurs a definite although small increase in the number of fat globules brought to the surface with ageing of the milk (19 hr. at 3–4° C.). Ageing seems to exert a slightly clumping action on the fat globules on the surface.

Table 5. *The effect of alcohol action as compared with the mechanical disturbance*

Milk sample	I, aged (26. i. 56)	J (10. i. 56)
Measuring temperature (° C.)	20–22	24
	No. of fat globules, clumps and lenses per sq.mm. of milk surface	
Milk surface after overflow	Very few	Very few
After 5 touches with platinum ring	1,340 globules and clumps	170 globules and lenses
After 10 touches with platinum ring	1,510 globules and clumps	880 globules and lenses
After 15 touches with platinum ring	—	1,010 globules, clumps and lenses
After 20 touches with platinum ring	2,180 globules and clumps	1,510 globules, clumps and lenses
Ethyl alcohol	1,640 globules and clumps	1,220 globules, clumps and lenses
n-Propyl alcohol	10,160 globules	5,920 globules, clumps and lenses
n-Butyl alcohol	11,680 globules and clumps	10,290 globules and lenses
n-Amyl alcohol	7,390 globules and clumps	—

Table 5 demonstrates comparative data obtained by the mechanical disturbance of the milk surface by touching with a platinum ring (diam. 3·5 mm.) and by the application of monohydric aliphatic alcohols. The effect of twenty touches is comparable with the effect of ethyl alcohol. Hence, the application of alcohols beginning with *n*-propyl alcohol is much more effective than the mechanical action.

The trihydric alcohol glycerol, with its three hydroxyl groups and a weak hydrophobic residue, is poorly adsorbable on the fat globules and accordingly gives a very slight rising effect. Substitution of one of the hydroxyls with a hydrophobic butyl group, as in monobutyrin, imparts the polar character to the molecule, and is accompanied by a strong hydrophobizing effect on the fat globules. When, however, all three hydroxyls are substituted by butyl groups, as in tributyrin, the clean-sweeping action on the milk surface takes place.

The addition of tributyrin to a milk surface crowded with fat globules through the action of monobutyrin sweeps this surface clean. On the other hand, the addition of monobutyrin to a milk surface swept clean through the action of tributyrin brings the fat globules to the surface.

B. *Surface-active substances sweeping the milk surface clean*

Since some members of Spans and Tweens, sorbitan and polyoxyethylene sorbitan esters of higher fatty acids have been used in studies on the dispersibility of whole milk powder (Hibbs & Ashworth(1); Mather & Hollender(7); Hollender(2)), it seemed of interest to investigate their behaviour towards fat on the milk surface. While the named authors made use of Span 40, Span 60, Span 62, Tween 60 and Tween 81, in the present investigation all members of Spans and Tweens were used.

Table 6 summarizes the observations on these surface-active agents. The agents in the form of powder or of waxy solid were dispersed in hot water and a loopful of this dispersion applied to the surface.

According to these data the liquid Spans and Tweens exhibit a pronounced sweeping action on the milk surface, the fat globules and clumps being pushed to the periphery of the surface, whereas the solid preparations are not so active. In some cases the spread-out surface-active agent was visible under the microscope, as a perforated, partly bright, partly iridescent layer. On standing, the fat globules do not reappear on the clean-swept surface, except with Span 20.

Spans and Tweens are non-ionic surface-active agents. Of cationic agents two were investigated, cetyl pyridinium chloride and cetyl trimethyl ammonium bromide. Like anionic sodium oleate, those cationic agents swept the milk surface clean.

DISCUSSION

When an alcohol inducing rising of fat globules is applied to the milk surface, it spreads out in a thin film, and some of it will dissolve in the milk immediately below the surface. The very poorly soluble higher members of the homologous alcohol series form a very thin surface layer interspersed with lenses containing the excess of alcohol. A part of the dissolved (possibly also spread out) alcohol molecules is taken up by the fat globules, and interacts with the globule membrane, with the result that the globule surface acquires water-repellency and the globules break through the milk surface. The hydrophobizing effect, as expressed by the number of globules coming to the surface, varies with different alcohols. Although methyl, ethyl and *n*-propyl alcohols are all completely miscible with water, the hydrophobizing effect of the latter is several times higher than that of the two former. This is presumably due to a certain optimum value of the length of the hydrocarbon chain, which gives the molecule a high hydrophobizing effect, yet does not impair its

solubility in water. With increase in the chain length the water-solubility of alcohols is appreciably reduced, their hydrophobizing effect is, however, only insignificantly changed (Table 1).

The simplest explanation of the hydrophobization of the fat globules by alcohols would be the formation of an adsorption layer of alcohol on the outer layer of membrane protein, the polar hydroxyl groups of alcohol linked to the polar side-chains of the protein, and its

Table 6. *The clean-sweeping action of Spans and Tweens on a milk surface containing moderate number of fat globules and small clumps* (500–700 *per sq.mm.*)

(Temperature 20–22° C.)

Trade name	Composition	Form at 25° C.	Behaviour on the milk surface
Span 20	Sorbitan monolaurate	Oily liquid	Larger part of surface swept clean; after some minutes the fat globules reappear on the surface
Span 40	Sorbitan monopalmitate	Fine crystalline powder	Surface swept clean patchwise
Span 60	Sorbitan monostearate		A comparatively small surface area swept clean; after an hour no reappearance of fat globules
Span 62	Sorbitan monostearate		A small surface area swept clean
Span 65	Sorbitan tristearate		Very slight sweeping action
Span 80	Sorbitan mono-oleate	Oily liquid	Larger part of surface swept clean; no reappearance of fat globules after ½ hr.
Span 85	Sorbitan trioleate	Oily liquid	Larger part of surface swept clean; large bright and iridescent areas of the agent; no reappearance of fat globules after ¾ hr.
Tween 20	Polyoxyethylene sorbitan monolaurate	Oily liquid	Larger part of surface swept clean; no reappearance of fat globules after ½ hr.
Tween 21	Polyoxyethylene sorbitan monolaurate	Oily liquid	Larger part of surface swept clean; a bright perforated layer of agent on clean-swept surface; no reappearance of fat globules after ½ hr.
Tween 40	Polyoxyethylene sorbitan monopalmitate	Oily liquid	A large part of surface swept clean; no reappearance of fat globules after ¼ hr.
Tween 60	Polyoxyethylene sorbitan monostearate	Liquid containing crystals	
Tween 61	Polyoxyethylene sorbitan monostearate	Waxy solid	A smaller surface area swept clean; no reappearance of fat globules after ¼ hr.
Tween 65	Polyoxyethylene sorbitan tristearate	Waxy solid	
Tween 80	Polyoxyethylene sorbitan mono-oleate	Oily liquid	Larger part of surface swept clean; no reappearance of fat globules after ¼ hr.
Tween 81	Polyoxyethylene sorbitan mono-oleate	Oily liquid	Larger part of surface swept clean; a perforated bright and iridescent layer of agent on clean-swept surface; no reappearance of globules after ¼ hr.
Tween 85	Polyoxyethylene sorbitan trioleate	Oily liquid	Very large part of surface swept clean; a perforated bright and iridescent layer of agent on clean-swept surface; no reappearance of fat globules after 1½ hr.

hydrophobic hydrocarbon chains orientated outwards. While this may be so with the higher alcohols, it is possible that with the C_3 to C_5 members deeper changes, viz. disruption of the lipoprotein linkages, are involved. In such an instance the alcohol would penetrate into the membrane and disengage the linkages between the hydrophilic groups of the phospholipid and protein. While the long fatty acid chains of the phospholipid remain anchored in the fat phase of the globule, probably interlocked between the fatty acid chains of higher melting fats (cf. King(5)), the hydroxyl group of the alcohol is attached to the hydrophilic group of the phospholipid, with the hydrocarbon chain orientated towards the plasma. The alcohol may interact in a similar way also with the hydrophilic side chains of protein liberated on the cleavage of lipo-protein linkages. On the other hand, the disruption of the lipo-protein may cause a mechanical cracking of the

membrane, with the result that some of the liquid fat content of the globule spreads out on the globule surface, rendering it hydrophobic.

Morton (8) found that the lipo-protein complex which binds milk alkaline phosphatase may be disrupted by treatment of milk or its products with *n*- or *iso*-butyl alcohol, while Hurst (3) observed the specific ability of *n*-butyl and *n*-amyl alcohols to disrupt the polar linkages between the lipid and protein in the lipo-protein from insect epicuticle. It was suggested that the alcohol competes for the polar side-chains of the protein, replacing removed lipids.

With the higher alcohols the hydrophobization of the fat globule surface enables the globules to concentrate in the lenses of alcohol on the milk surface.

Hydrophobization of the fat globule surface may be regarded as the first stage in the demulsification of milk fat, which—when there is a sufficient amount of the liquid fat fraction available—leads to the cementing together of the fat globules, or clumping. The final stage of demulsification is the breaking of the fat-in-plasma emulsion. In several methods for the volumetric determination of milk fat, propyl, butyl, and amyl alcohols, usually their *iso*-forms, are used to promote this breaking of the emulsion.

According to Patton (9) the optimum number of carbon atoms in an alcohol for acting as a demulsifying agent for milk or cream was 3 to 5. Thus, *n*-propyl, *iso*-propyl, *n*-butyl, *sec.*-butyl, *tert.*-butyl, *iso*-amyl alcohols were effective agents, heptyl alcohol was partially effective, whereas *n*-octyl alcohol was ineffective. An aqueous reagent containing *n*-butyl alcohol and *n*-butylamine was capable of demulsifying a number of fluid dairy products, which gave a rapid and convenient means of preparing pure milk fat for the determination of fat constants, for studies of its composition, etc.

Some of the synthetic surfactants Spans and Tweens, which belong to the surface-active agents sweeping the milk surface clean of fat, have been used to improve the dispersing characteristics of whole milk powder. Since some of these agents at certain concentrations cause churning of the fat when the powder is reconstituted, a proper balance between the hydrophilic and lipophilic groups of agents seems to be necessary to obtain a whole milk powder that is self-dispersible, but still does not churn when reconstituted (Mather & Hollender (7)). The more of a hydrophilic compound that is present the higher the self-dispersibility, but also the higher the tendency to churn. The addition of a more lipophilic compound will reduce the self-dispersion, but control the churning.

In the light of the present observations, it seems possible that on spraying the milk in the presence of a Span or Tween, the surfactant, apart from being taken up by the surface membrane of fat globules, spreads out on the surface of the freshly formed milk droplets and sweeps their surface clean of the fat which otherwise would impair the wettability and thus also the dispersibility of the obtained powder particles. On agitation during reconstitution there occurs—depending upon the balance of hydrophilic and lipophilic groups—a rearrangement of the surfactants on the globule surface, which may become predominantly hydrophobic, and clumping of the globules may take place.

SUMMARY

Surface-active substances applied to the surface of whole milk may either bring fat globules, clumps, lenses and patches on to this surface, or sweep the surface clean of these fat forms.

In the homologous series of monohydric aliphatic alcohols, methyl and ethyl alcohols show a moderate rising action, whereas the other members from *n*-propyl alcohol to *n*-decyl alcohol are strongly active. There is only a slight difference in the activity of primary, secondary and tertiary butyl alcohols.

Ageing of milk slightly promotes the rising of fat globules on to the surface under the action of ethyl to *n*-amyl alcohols.

Alcohols, beginning with *n*-propyl alcohol, bring fat globules to the surface of milk much more strongly than does mechanical disturbance of the surface by touching with a platinum ring.

Glycerol, due to its three hydroxyl groups and a weak hydrophobic residue, is almost inactive, monobutyrin is strongly active in bringing the fat globules to the surface, while tributyrin sweeps the surface clean.

Spans and Tweens, sorbitan and polyoxyethylene sorbitan esters of higher fatty acids sweep the milk surface clean.

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653. A CALORIMETRIC STUDY OF MILK, CREAM AND THE FAT IN CREAM

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(With 9 Figures)

INTRODUCTION

The fat in milk consists of a complex mixture of a number of glycerides which have different melting-points, and are probably capable of exhibiting mixed crystal formation (1). It is characteristic of a mixture of this type that it melts and solidifies over a range of temperature; the phase transitions are known to be influenced by the physical condition of the fat and the manner in which they are effected. That the process is also intimately associated with the intersolubilities of the various glyceride components is not to be doubted. Indeed, there would appear to be no particular distinction between melting and solution (2).

Hofgaard (3), Bailey (4) and co-workers and many others have demonstrated the application of dilatometric and calorimetric techniques to investigations of phase transitions in commercial plastic fats and oils. Thermal analysis involving heating or cooling curves has also proved useful in the examination of polymorphism in fats. Dilatometry is admirably suited to qualitative appraisals of melting and solidification processes at other than very low temperatures, requiring only simple apparatus and small samples. Being a static method, long-term phase transitions can be followed easily and accurately. The technique has found applications in the study of the thermal behaviour of dairy cream and butterfat (1,5,6,7,8).

Calorimetric analysis of phase transformations involves rather more elaborate apparatus and procedure. The method has been used for studies of milk and cream by a number of workers, but certain qualitative aspects of the crystallization behaviour of the milk fat have not been fully appreciated. This, together with the application in some instances of calorimetric methods of doubtful suitability, has led to a diversity of quantitative data.

This paper describes a calorimetric investigation of the thermal behaviour, and particularly the specific heat/temperature relationships, of milk, cream and milk fat in the temperature range 0–50° C. This is the region in which the major latent heat effects associated with the melting and solidification of the fat are encountered.

PREVIOUS CALORIMETRIC WORK

The early work on the specific heat of milk and milk derivatives includes that of Fleischmann (9), who used a method of mixtures, Schnorf (10) (Bunsen ice calorimeter), and others (11). Little information regarding the variations of the specific heat with temperature appears to result from these researches, and discussion of this very early work is omitted in favour of the more recent investigations.

The work of Hammer & Johnson (12) with an electrically heated calorimeter, led to specific heat/temperature curves for milk and creams, but their method appears to have suffered from lack of precision, and their generalized results have obscured the true behaviour of the fat. Bowen (13), in discussing refrigeration techniques for milk, presents some data obtained by the National Bureau of Standards. The experimental method is not mentioned (although electrical heating was probably employed), and the preconditioning of the milk and cream is not fully described. His cream curves, however, resemble those subsequently obtained by Rishoi & Sharp (14). These workers used both the calorimetric and dilatometric (6) techniques to follow the phase changes in the fat of rapidly cooled cream of about 40% fat content. An important outcome of their work is that, when ordinary cream is cooled rapidly and maintained at a temperature within the crystallization range of the fat, thermal equilibrium of the globular fat takes substantially 4–5 hr. Small, long-term adjustments can apparently take place after this, continuing for days and possibly months until, presumably, complete thermal equilibrium is attained. However, considerations of absolute equilibrium appear to have little practical significance, and do not enter this discussion. In view of Rishoi & Sharp's findings, it is understandable that attempts (15) to determine transition points of milk fat by means of cooling curves, met with little success.

More recent, but limited, specific heat data have been obtained by Jack & Brunner (16) in their study of the relation between the degree of fat solidification and churning time of cream. They used an electrically heated calorimeter capable, it would seem, of an accuracy of about 2%.

Mikhailov (17) reports some specific heat values for dried skimmed milk and milk fat.

SCOPE OF THE PRESENT INVESTIGATION

It was thought a worthwhile preliminary to re-examine the phenomenon of delayed fat crystallization in cooled cream, in view of its importance when considering the treatment procedure prior to calorimetric measurements. This was done with a simple method of mixtures similar to that of Rishoi & Sharp. The results of these experiments, and of the application of the particular method to determining specific heat/temperature curves of the fat in cream, are given.

Using an adiabatic type of calorimeter especially constructed for this work, a more detailed examination in the range 0–50° C. was subsequently made of the specific heat/temperature variations of separated milk, whole milk, cream (15% fat content) and, derived from the latter, the milk fat. The effects of slow, rapid and step-wise cooling, and different temperature/time variations as pretreatment factors, were studied.

PRELIMINARY EXPERIMENTS

Delayed fat crystallization in cooled cream

Using a method and procedure differing only in detail from that of Rishoi & Sharp, their observations regarding the delay in crystallization of the fat in ordinary cooled cream were confirmed. Only brief mention is made of the experimental procedure and the effects illustrated by the results from a single typical experiment.

A quantity of thick cream (about 50% fat) obtained from mixed milks, was pasteurized

and rapidly cooled to 5° C. in a commercial processing plant and then maintained at this temperature. Samples were taken of this periodically and their average specific heats determined over the range of temperature required to melt the fat (i.e. 5–45° C.). The principle of the method consisted of pouring a quantity of the cooled cream into a calorimeter containing hot water at a known temperature. With corrections for heat losses, the usual measurements and calculations enabled average specific heats to be determined. The specific heat of water was taken as unity, and heats of mixing assumed absent. The accuracy of these determinations was about 1%.

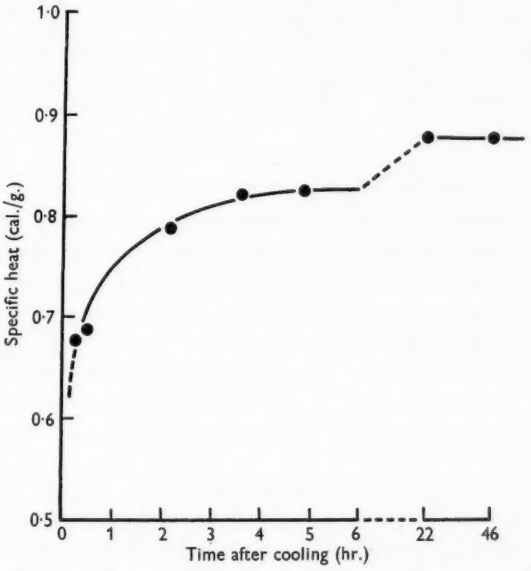

Fig. 1. The change in the specific heat of the fat in ordinary cream after rapid cooling to 5° C. from the pasteurization temperature.

It was assumed that the cream was composed of two components, milk fat and separated milk, and further, that the latter had the same composition as the separated milk from the original cream separation. The specific heat of this separated milk was determined over the same temperature range as that for the cream samples. These data, and a knowledge of the percentage of the components present, enabled the average specific heats of the fat in each cream sample to be calculated. Taking into account the derived errors in computation, the overall accuracy of the values for the fat is of the order of 2–3%, depending on the actual specific heat value.

A plot of the specific heat of the fat against time after cooling is shown in Fig. 1. Immediately after cooling, the specific heat was low, indicating that little or no fat had solidified. As time elapsed, so the specific heat increased, showing that solidification was proceeding. After about 4 or 5 hr., a near-maximum had been reached, showing that the majority of the fat capable of crystallizing at the storage temperature (5° C.) had done so. A subsequent slight rise in specific heat took place very slowly, until a constant value, indicative of a state of complete equilibrium, was attained.

Density/time measurements in this laboratory on cooled whole and enriched milks (18), together with some experiments to be described on 15% fat cream, corroborated these findings, as well as showing that the effect is independent of the fat concentration. Approximately the same times to reach equilibrium were recorded when cooling to other temperatures between 0 and 20° C. Thus, although less fat would be capable of crystallizing at higher temperatures, the time for equilibrium appeared to be practically independent of the degree of cooling.

It is important to note that the above observations were made on cream separated from milk in the ordinary way. Differences in the rate of crystallization of the fat in homogenized and ordinary cream, however, have been noticed by Van Dam (7), followed by Mulder (1), therefore showing that the degree of dispersion of the fat is an important consideration. Their conclusions, together with confirmatory results from some calorimetric and other experiments to be described later, constitute convincing evidence of the effects of fat globule size.

Specific heat/temperature curves for milk fat

The method of mixtures was again used in these experiments. A quantity of ordinary cream (~50% fat), separated from mixed milks, was warmed to about 45° C. to melt the fat. Samples were then taken and cooled, one to each of the nominal temperatures 0, 5, 10, ..., 30° C., by immersing their containers in appropriate water-baths. They were stored at these temperatures overnight (~16 hr.). The average specific heats of the cream in the temperature ranges 0–45, 5–45, 10–45° C., etc., were then determined. Measurements of the specific heat of the separated milk component over the same temperature intervals then permitted calculations of the average specific heat of the fat in 5° C. intervals, viz. 0–5, 5–10° C., etc. In practice, final mixture temperatures of exactly 45° for the cream and water could not generally be brought about, the deviations being of the order of 1° C. However, determinations of the average specific heat of melted fat around 45° C. enabled corrections to a constant final temperature to be readily made.

The results with this cream treatment are plotted in Fig. 2, curve *A*, and can be compared with curve *B*, taken from the data of Rishoi & Sharp (14).

There is good agreement up to 12° C. and between 25 and 35° C., but their high value at about 18° C., being the peak latent heat effect, has not been reproduced. Curve *A* shows a peak value of approximately 1·5 cal./g. around 15° C.

A modified form of cream treatment led to curve *C*. After warming the cream to 45° C., *all* the samples were cooled rapidly and stored at 0° C. overnight. Subsequently, they were *warmed* to their respective temperatures 5, 10, ..., 30° C. and their specific heats measured as before. It will be seen that in curve *C*, the temperature at which the maximum occurs is the same as in curve *A*, but distinctly lower values of specific heat are evident between 0 and about 15° C.; moreover, higher values above 25° C. are now noticeable. The results represented by curves *A* and *C* were reproducible to approximately 2%.

These comparatively rough experiments served to show that the manner of preparation of the cream influenced the crystallization behaviour of the fat. Unfortunately, the simple method of mixtures is limited in its application and, in this case particularly, is not well suited to a detailed examination of the specific heat variations produced by various cream pretreatments. The study was continued, therefore, using an adiabatic method which enabled hitherto undetected features of the specific heat curves to be resolved.

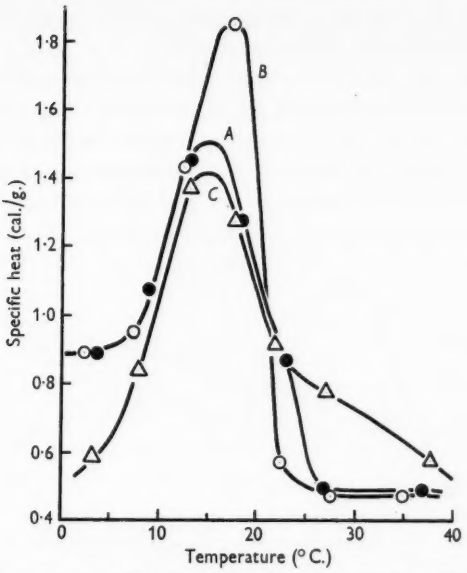

Fig. 2. Specific heat/temperature curves of the fat in cream obtained with the method of mixtures. *A*, ●—●, cream samples directly cooled to initial temperatures; *B*, ○—○, similar treatment, Rishoi & Sharp (1938); *C*, △—△, cream samples initially stabilized at 0° C.

ADIABATIC CALORIMETRY

The adiabatic type of calorimeter used in the following experiments has been fully described elsewhere (19). This apparatus enabled specific heats within small temperature intervals (2 or 3° C.) to be measured with an accuracy of ±0·5% over its full working range (0–100° C.), and rather better than this (±0·3%) between about 10 and 30° C.

The milk or cream for test (~125 ml.) was contained in a thin, stainless steel calorimeter supported inside a co-axial, temperature-controlled jacket, which itself was situated in an enclosure whose temperature could be controlled. A novel form of stirring was used, involving an oscillation of the whole assembly about its vertical axis. This action, in association with fixed metal fins inside the calorimeter, promoted stirring of the liquid. Adiabatic conditions could be maintained whilst a measured quantity of heat was supplied to the calorimeter, the resulting temperature rise being measured (to 0·002° C.) with a ten-junction thermoelement. The calorimeter heater was of a design which gave wide dispersion of heat without local overheating. The effect of different rates of heating was the subject of a separate investigation, the choice of a suitable rate being particularly important when examining cream. Temperature/time graphs following the cessation of too high a heating rate, indicated that an 'overmelting' of the fat could occur, followed by slow resolidification. It was important that this should not occur.

SPECIFIC HEAT OF SEPARATED AND WHOLE MILK

Fig. 3 shows a plot of the specific heat against temperature of separated milk (about 0·05% fat, 9·2% S.N.F.), obtained from bulked morning and evening milk, which had been stored at 0° C. overnight in the calorimeter packed in ice. For all practical purposes

the relation can be taken as linear, the effects due to the presence of the very small quantity of fat being imperceptible. Pasteurization, or any of the thermal pretreatments later used for creams, produced no modifications.

Taking the specific heat of water as unity, the apparent specific heat of the S.N.F. at various temperatures can be calculated from these results, some particular values being 0·45 cal./g. at 40° C., 0·37 at 20° C. and, by extrapolation, 0·27 at 0° C. Specific heat curves for separated milk with different S.N.F. contents can therefore be determined. However, the variations in the concentration, and composition, of the S.N.F. commonly

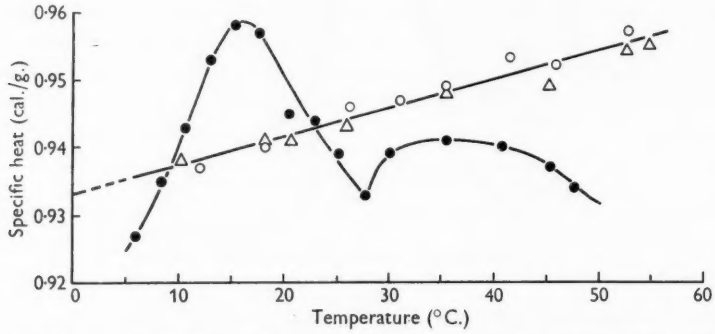

Fig. 3. Specific heat/temperature curves for separated milk and whole milk. ○—○, separated milk, <0·1% fat, 9·2% S.N.F.; △—△, repeat run; ●—●, whole milk, 3·96% fat, 8·82% S.N.F.

encountered in the separated milk from bulked milks are small, and would not normally cause significant deviations from the results in Fig. 3. Accordingly, as all cream samples in the later work were obtained from bulked milks, these values were used in calculations of the contribution of the separated milk to the specific heats of cream when deriving data for the fat alone.

It should be noted that the principle of the additivity of specific heats is invoked throughout, although, in view of the presence of water-soluble components of the S.N.F., this may not strictly be true. As the percentage of these components involved is very small, however, the validity of the assumption need hardly be questioned. Moreover, the general agreement found between the specific heats of separated milk obtained by the adiabatic and the mixtures methods gives added confidence in its validity, certainly where small percentages of S.N.F. are concerned.

Table 1 enables a comparison to be made of representative data for separated milk. Generally speaking, there is satisfactory agreement between workers, although the accuracy implied in some cases by tabulation of the third significant figure is questionable, especially where S.N.F. percentages have been omitted.

The different value of J, Joules's equivalent, used by Hammer & Johnson in their electrical method, should be noted. For $J = 4{\cdot}186$ joules/cal. (20), their figures would be higher by rather more than 1·5%.

The specific heat/temperature curve for a typical whole milk is also shown in Fig. 3. A sample taken from bulked morning and evening milk (3·96% fat, 8·82% S.N.F.) was pretreated by warming to about 45° C. to melt the fat, and stored overnight at 0° C. in the

calorimeter packed in ice. By storing at 0° C., practically complete solidification of the fat was ensured, and latent heats of melting were expected to be a near-maximum.

Phase changes of the fat are evident around 15° C. and again about 35° C. The magnitudes of these effects are dependent upon the fat concentration and the thermal history of the sample. As the former is low in milk the effects are not prominent. This can be gauged from a comparison with the separated milk curve, the difference between values at corresponding temperatures being generally less than 2%.

Table 1. *The specific heat of separated milk (cal./g./° C.)*

Composition (%)		Temperature (° C.)						
Fat	S.N.F.	0	10	20	30	40	50	Investigator
0·2	—			0·9455 (27·5°–40°)				Fleischmann
0·2	—			0·9388 (14°–16°)				Fleischmann
0·30 to 0·38	—	0·940	0·943 (15°)		—	0·952	—	Hammer & Johnson (J = 4·26 joules/cal.)
				← Average value 0·949 →				Hammer & Johnson (J = 4·26 joules/cal.)
—	—	←		Average value 0·943		→		Rishoi & Sharp
—	—	0·943	0·944	0·946	0·949	0·954	0·960	Jack & Brunner (J = 4·186 joules/cal.)
< 0·1	9·2	0·933*	0·938	0·942	0·946	0·950	0·954	Phipps (J = 4·186 joules/cal.)
0·02	9·0		←	Average value 0·948		→		Phipps (method of mixtures)

* Extrapolated.

Milk may be regarded as a particular example of a cream. As the data on cream and milk fat in the next section enables curves to be obtained with fair accuracy for milk of different fat concentrations and thermal pretreatments, the study of milk itself was not continued.

Only a few workers have obtained data for whole milk. These, in some instances, have taken the form of isolated values, and in others, averages over certain temperature intervals. The variations anywhere between 0 and 50° C. are admittedly small, and for some purposes no great error would be occasioned by taking mean values over chosen temperature intervals. This can be done with the results in Fig. 3. Bowen's results (13) are probably worth mentioning, as a definite specific heat/temperature relationship is presented. Nevertheless, the method of measurement and the thermal pretreatment of the samples have not been discussed. On the whole, the value of most of these earlier results has been lessened by the lack of information on the experimental conditions and procedure.

In view of the doubts attached to these evaluations, a tabular comparison has not been made.

SPECIFIC HEAT OF CREAM AND MILK FAT

When referring to cream, it is convenient here to imply fat contents of greater than about 10%.

A prerequisite for accurate calorimetric measurements on liquids is, usually, adequate stirring. The fat contents of the creams chosen for examination were governed in the first instance by the ease with which the cream could be stirred at low temperatures. An optimum fat content of approximately 15% was found to fulfil this requirement and, at the same time, allowed reasonably accurate calculations of the specific heats of the fat itself to be made.

In all these experiments, the cream was obtained from raw, bulked, morning and evening milk by the normal dairy separation methods, the separator being arranged to deliver cream of about 20% fat content. Adjustment to about 15% fat content was then made by remixing this carefully with a suitable quantity of the original separated milk. Gerber tests were used for the fat percentage evaluations.

It was usually found advisable to pasteurize creams for those protracted experiments where the keeping quality might have been in doubt. Heat processing above 50° C. generally was found to produce no noticeable alterations in the subsequent specific heat characteristics of cream between 0 and 50° C., provided that, as a consequence, there was no substantial change in the average globular size of the dispersed fat.

In view of the linear relationship for the separated milk component (Fig. 3), specific heat curves for cream reflect, principally, the behaviour of the fat. For this reason, and the fact that the fat percentages of the cream samples were not precisely the same, the curves for the fat, rather than for the cream, are presented. This permits a more accurate comparison of the effects of the different treatments as well as allowing direct comparison with the results of the earlier experiments.

As the fat is dispersed in discrete globular form, and not in solution, in the separated milk, the cream can reasonably be regarded as a simple mixture of these two components, and it is highly probable that the specific heats are additive. No experimental evidence was found to suggest that the relation was otherwise, and calculations were consequently made with this assumption.

The magnification of errors in computing the specific heats of the fat from the corresponding ones for the 15% creams leads to an estimated maximum possible error of about 2% for the fat figures.

(i) *The influence of storage temperature*

After cooling and maintaining an ordinary cream at a chosen temperature, it was seen earlier that a definite minimum time was required for the fat to reach a reasonably stable condition. This time for equilibrium appeared to be practically independent of the storage temperature. However, the following experiments show that changes can be brought about in the specific heat curves of a stabilized cream by varying the temperature at which stabilization is effected.

The pretreatment of all test samples of cream in these experiments included an initial warming to about 50° C. in order to melt the fat and ensure reproducible starting conditions. The procedure then consisted of filling the calorimeter with the warm cream, sealing off with its lid, weighing, and immersing in a water-bath at the selected storage temperature. The storage times were of the order of 16 hr., that is, well in excess of the minimum time for effective equilibrium. The time to cool to a selected temperature between 0 and 20° C. was found to be approximately 10–15 min. This cooling rate can be regarded as rapid in contrast to the slow cooling deliberately induced in some later experiments.

A number of specific heat curves for the fat, corresponding to different storage temperatures, were obtained, but to avoid confusing the diagrams, only three have been selected to illustrate the behaviour. Before discussing these results as a group, however, reference is made to Fig. 4, which illustrates the order of specific heat values obtained for cream of about 15% fat. Here, the curve for cream and the derived one for its fat, for a storage

temperature of 0° C., are shown. The similarities of their shapes are evident, but whereas the values for the fat range from 0·67 cal./g. at 5° C. to 1·52 at 15·5° C. and thence to 0·52 at 45° C., the corresponding cream values are 0·896, 1·025 and 0·890 cal./g.

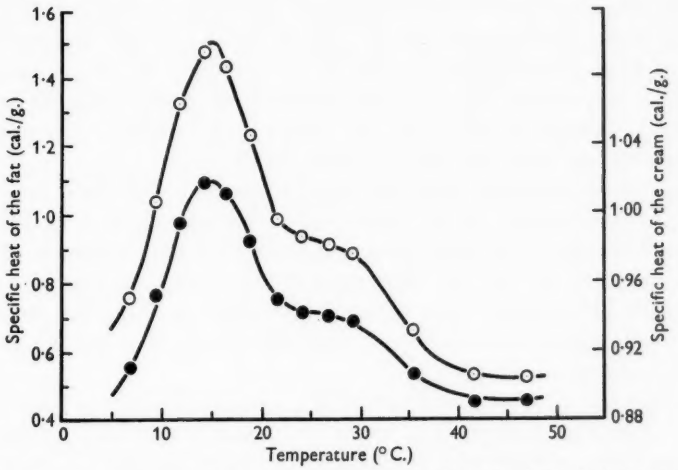

Fig. 4. Specific heat/temperature curve for 14·5% cream (●—●) and the derived curve for the fat alone (○—○) after storage at 0° C. for 16 hr.

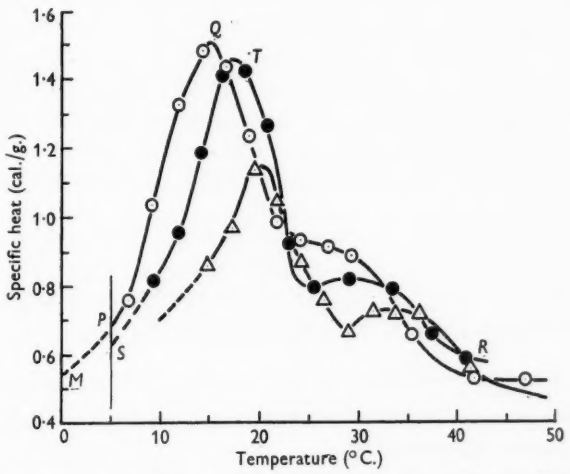

Fig. 5. Specific heat curves for the fat in cream cooled directly to, and stored at, different temperatures. ○—○, storage at 0° C.; ●—●, 5° C.; △—△, 10° C.

Turning now to Fig. 5, the group of curves for the fat corresponding to storage temperatures of the cream of 0, 5 and 10° C. are shown. Extrapolation over a few degrees back to the actual storage temperature was usually necessary in these graphs as the temperature of the sample unavoidably increased slightly during the assembling of the apparatus and before measurements could begin. It will be seen that the curves are

similar in character, each one exhibiting the familiar major peak followed by another minor one, sometimes only an inflexion, at higher temperatures. The major peak is attributable to the principal latent heat effect associated with the melting of the fat. With progressively higher storage temperatures, correspondingly reduced magnitudes of the effect are observed since less fat is capable of crystallizing at the higher temperatures. In addition, however, the position of the peak is shifted to higher temperatures. Thus, storage at 0° C. gives a maximum at 15·5° C., but for 5 and 10° C., the maxima are at 17·5 and 20° C. respectively. This progressive shift of the primary peak did not appear to be extended by storage temperatures much above 18° C.; moreover, for storage at this last temperature, the maximum only moved to about 21° C.

The above phenomenon suggests that different storage temperatures lead to crystallization of fat of different glyceride compositions. This inference was, in fact, drawn by Mulder (1) on the evidence of similar behaviour observed in his dilatometric studies. It is interesting to record that the curve between 0 and 40° C. for a sample stored at −30° C. was practically identical with that obtained by storage at 0° C., thus indicating that apparently no change in the composition of the solid fat could be brought about by low temperature holding.

Above about 25° C., after completion of the main melting effect, there are distinct indications of another phase change whose peak value is also moved progressively to higher temperatures with increasing storage temperature. The effect is noticeable in the '0° C.' curve as only an inflexion around 25–30° C., but in the last curve assumes a hump with a maximum at about 35° C. The onset of the phase change appears to be dependent upon the storage temperature and, as before, implies that fat of mixed crystal forms, capable of assuming different compositions, is involved. The effect, though small, suggests the presence of an 'independent' fraction of fat composed of glycerides of higher melting-points than the remainder. On the other hand, Mulder (1) has observed the behaviour of butter fat in this temperature region under the microscope, and found evidence to suggest the existence of a metastable form of the fat which melts around 30–40° C. It is possible, therefore, that the specific heat variations observed here are caused by the presence of this metastable form.

Two final points to mention in connexion with the above curves are: the specific heat of the wholly melted fat (45° C.) is about 0·52 cal./g.; the curve for a milk of 3·96% fat content, derived from the '0° C.' curve above and the separated milk results of Fig. 3, agrees well with that obtained by direct measurement (Fig. 3).

(ii) *Considerations of the equilibrium of the fat in warmed cream*

It is convenient here, where ready reference may be made to Fig. 5, to mention the behaviour of the fat in warmed cream.

Experiment showed that, analogous to the delay in crystallization of the fat in cooled cream, the attainment of equilibrium of the fat after *heating* a cream through a small temperature interval within the melting range was also not immediate. This bears out the conclusions drawn from dilatometric analysis (1). The effect was demonstrated calorimetrically by stabilizing an ordinary 15% cream at 0° C. for 16 hr., and then warming and holding at 10° C. for a further 16 hr. before proceeding, from this temperature, with specific heat measurements as usual up to 40° C. The resulting curve was found to have a smaller primary peak, at a temperature 2·5° C. higher (at 18° C.), than the '0° C.' curve

in Fig. 5, despite initial storage at 0° C. Thus, after 16 hr. at 10° C., measurable alterations in the proportions and composition of the solid and liquid fat phases took place. The question of the rapidity, and hence the influence on actual specific heat determinations of these equilibrium adjustments then arose, since intermittent heating of the cream was a necessary part of the experimental procedure.

Under normal working conditions, where local overheating was absent, the time for an ordinary 15% cream to reach apparent thermal equilibrium in the calorimeter after a heating period was about 10 min., temperature drift of the sample being then zero. The interval between successive experimental runs was ordinarily no more than a few minutes. Prolonging the intervals to about an hour produced no noticeable changes in the specific heat curve for a given pretreatment. Adjustments in the physical state of the fat were thus shown to be imperceptible for at least an hour after heating through intervals of 2 or 3° C. The immediate effects of this post-heating instability of the fat on specific heat determinations conducted in the usual manner were therefore negligible.

The curve for fat, which was initially in a melted state and then cooled and stored at 10° C. (Fig. 5), showed a maximum (1·16 cal./g.) at 20° C. but, as remarked above, this was at 18° C. (1·41 cal./g.) for fat initially solid and warmed to the same storage temperature. The melting and solidification processes are thus not wholly reversible in the storage times considered here. If the storage time had been increased, no doubt the curves resulting from the two treatments would have tended to coincide with an intermediate maximum value at a temperature between 18 and 20° C.

(iii) *A comparison of the results obtained with the mixtures and adiabatic methods*

A re-examination of the results obtained by the method of mixtures (Fig. 2) and a comparison with those in Fig. 5 can now be made.

In obtaining curve *C*, Fig. 2, samples initially at 45° C. were cooled and stabilized by keeping at 0° C., the fat being then mainly in the solid state. The samples were then warmed to the respective temperatures 5, 10° C., ..., etc. As the subsequent specific heat measurements were completed within an hour or two of the cream attaining these temperatures, the extent of the effects on warming, previously discussed, were small and unimportant. Curve *C* may be compared with the '0° C.' curve in Fig. 5. A consideration of the experimental procedures shows that, in the two cases, the thermal conditions imposed upon the cream by the pretreatment and subsequent process of measurement were closely similar. There is good agreement, both qualitative and quantitative, between the two curves.

The treatment of the cream resulting in curve *A* (Fig. 2) was rather different. Here, all samples were cooled directly to their respective temperatures 0, 5, 10° C., ..., etc., and was the procedure adopted by Rishoi & Sharp in obtaining curve *B*. The treatment leads to the relatively higher specific heat values between 0 and about 15° C. (The high peak value in curve *B* is considered likely to be erroneous.) The different results obtained in this temperature region with the two methods of preparation are a consequence of the lack of complete reversibility, during the particular storage time, of the melting and solidification of the fat. Thus, less fat solidifies at any one storage temperature by cooling than by warming to it from a stabilized condition at 0° C. Referring to Fig. 5, if point *P* on the '0° C.' curve is at 5° C., the areas under the curves *MPQR* and *PQR* bounded by the abscissa line at zero specific heat are equivalent to the quantities of heat required to

warm one gram of the fat from 0 to 45 and from 5 to 45° C. respectively. Their difference represents the heat required to warm the fat from 0 to 5° C., and hence an average specific heat value over this temperature range can be calculated and plotted at 2·5° C. Similarly, from the '0° C.' curve, values at 7·5, 12·5° C., etc., can be obtained. This procedure is, in fact, the basis of the measurements in the mixtures method, and with samples initially stabilized at 0° C. and brought up to their 'starting' temperatures, yields curve *C* of Fig. 2, which, as noted earlier, is in good agreement with the '0° C.' curve over the complete temperature range.

Consider now, cooling directly to 5° C. The heat required to warm from 5 to 45° C. now appears to be equivalent to the area under the curve *STR*. The difference between areas *MPQR* and *STR* is greater than between *MPQR* and *PQR* and so a larger specific heat is calculated for 2·5° C. than before. Likewise, consideration of the '5° C.' and '10° C.' curves yields a higher value at 7·5° C. The differences between curves *A* and *C* from 0 up to about 15° C. can be accounted for in this way.

The minor phase change observed in 'adiabatic' measurements between about 25 and 35° C. is noticeable in curve *C* but not in *A*. It would thus appear that cooling below some limiting storage temperature is required for this effect to be produced.

From a series of curves such as those in Fig. 5, it should be possible to construct a single specific heat/temperature curve, following the form of curve *A*, by the method outlined above. Although this was, in fact, attempted, the accumulated errors arising from experiment and calculation did not permit accurate measurements to be made of the areas under the curves.

(iv) *The influence of variations in the cooling pretreatment*

The experiments so far have been concerned with relatively rapid cooling of the cream. However, for a given temperature and time of storage, slow cooling, interrupted or stepwise cooling, and tempering, which involves a cooling-warming-cooling cycle, are all factors which influence the specific heat curves of the fat by modifying its crystallization behaviour. The results of experiments illustrating the effects of these variations in the cooling pretreatment are shown in the accompanying figures.

Slow cooling. The curve for the fat resulting from relatively slow cooling to 0° C. is shown in Fig. 6. After warming to 45° C., the cream sample was cooled to 30° C. by immersing the calorimeter in a water-bath at that temperature, and then at hourly intervals thereafter, the temperature was reduced by 5° C. until at 0° C. it was stored for 16 hr. as usual.

This slow-cooling treatment leads to a curve of similar shape to the one obtained by rapid cooling (reproduced here for comparison), with the maximum occurring at 15·5° C., but of a reduced magnitude. The diminished area under the curve indicates that less fat solidified under the conditions of slow cooling.

Interrupted cooling. The effect of introducing a single, prolonged interruption in cooling is also shown in Fig. 6. Here, the calorimeter containing the warm sample was immersed in a water-bath at 10° C. for 5 hr., and subsequently transferred to the ice-bath for 16 hr. It will be seen that temporarily arresting the cooling at 10° C. has introduced some asymmetry into the curve over the main melting range, and, despite final storage at 0° C., the maximum occurs at 19 or 20° C. This practically coincides with the temperature of the maximum obtained by simply cooling and stabilizing at 10° C. (see Fig. 5), and

indicates the similarity in the composition of the solid fat in the two cases. From a consideration of the areas under the curves, the amount of fat solidified by the interrupted cooling treatment was even less than for slow cooling.

It might have been expected that, after holding at 10° C., cooling and storage at 0° C. would have resulted in the maximum being at a lower temperature, and of greater magnitude. The fact that this did not occur shows, as Mulder (1) infers, that the solid and liquid fat phases interact only with difficulty. Thus, in this case, the fraction of fat solidified by cooling to 10° C. becomes virtually isolated, and neither influences, nor is influenced by,

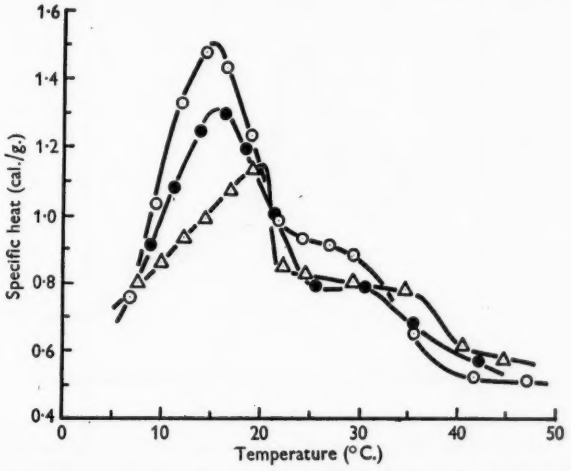

Fig. 6. The influence of different modes of cooling upon the specific heat curves of the fat in cream. ○—○, rapid cooling to 0° C.; ●—●, slow cooling to 0° C.; △—△, cooling to 0° C. interrupted at 10° C. for 5 hr. In each case, final storage at 0° C. was for 16 hr.

the solidification of the fats of lower melting-points on further reduction of the temperature. This 'separation' of fat fractions is evident in the experiment that follows.

Tempering. A thermal treatment taking the form of a cooling-warming-cooling cycle, is in the nature of a tempering process. This process is one often employed commercially for improving the consistency and other properties of some of the plastic fats. In practice, the fat is solidified by being cooled rapidly to a low temperature, and then tempered by warming to an intermediate temperature before finally cooling again. The curves for the fat in a cream treated in this fashion are shown in Fig. 7. Curve (*a*) is for a sample which, after warming to 45° C., was cooled and maintained at 0° C. for 16 hr., then warmed and kept at 15° C. for 1 hr., and finally stored at 0° C. for a further 16 hr. This treatment produces a double peak within the main melting range, one occurring at about 9° C. and the other at 18° C. Maintaining at 15° C. for a longer period, viz. 8 hr., led to curve (*b*) and shows the accentuation of the first smaller peak at the expense of the second, larger one. There is also a shift of the whole curve with respect to (*a*), with the maxima now occurring at about 10 and 19° C. Actually, the positions of the smaller peaks are not particularly well defined with the few points available, but the general pattern is apparent. Beyond 25° C., the curves are virtually coincident.

The explanation of the observed effects appears to be that, on warming the cold cream to 15° C., the fats of lower melting-points are melted, and on cooling again they solidify but retain their identity. Their presence is then indicated on the subsequent specific heat curve by the latent heat effect around 9 or 10° C. Maintaining for the longer time at 15° C. (curve (*b*)) allows a greater fraction of the fat to 'separate out', and thus its latent heat effect is enhanced. A corresponding reduction in the latent heat of melting of the fats at the higher temperature (around 18° C.) is then observed. The shift of the maxima with increasing time at 15° C. shows that complementary adjustments in the composition of the solid and liquid fat phases take place.

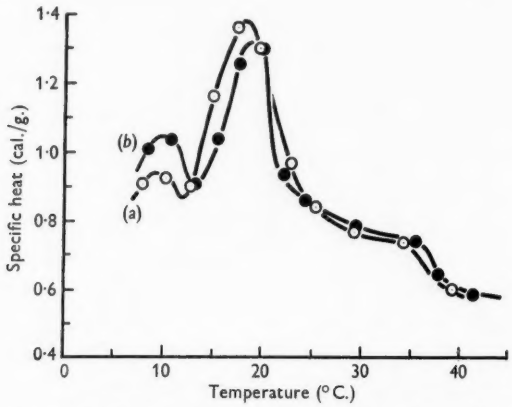

Fig. 7. Specific heat curves of the fat in cream subjected to a form of tempering treatment. (*a*), ○—○, stabilized at 0° C. for 16 hr., warmed and kept at 15° C. for 1 hr., finally stored at 0° C. for 16 hr. (*b*), ●—●, similar treatment, but 8 hr. at 15° C.

It would appear that a process such as tempering, which leads to distinct multiple peaks, or what amounts to fractional crystallization, in fact leads to decreased mixed crystal formation.

(v) *The influence of the degree of dispersion of the fat*

Van Dam (7), using a dilatometric technique, demonstrated that the fat in homogenized cream solidifies less readily than in ordinary cream. It would thus appear that the degree of dispersion of the fat in cream influences the crystallization rate. Others (1, 21) have shown too that dispersed fat, in turn, is less readily solidified than in the non-dispersed or continuous form.

Although it was not possible to examine the behaviour of butter-fat in the continuous state with the present calorimetric arrangement, the retarded solidification of the fat in cream as the result of increased dispersion is clearly evident from the following experiment. An ordinary cream of about 15% fat content was heated to about 70° C. and then passed three times through a small, manually operated homogenizer. A microscopic examination of the cream before and after homogenizing showed that a higher degree of dispersion of the fat had been effected by the process. After ensuring that the fat was in the wholly melted condition, a sample of this homogenized cream, contained in the calorimeter, was immersed in melting ice and stored for 16 hr. The subsequent specific

heat curve of the fat shown in Fig. 8 may be compared with the '0° C.' curve (Fig. 5) for the fat of an ordinary cream which had received identical cooling treatment. The smaller area under the main peak of the former curve, together with the fact that the maximum is at 17·5° C. as compared with 15·5° C. shows that, even after 16 hr., the homogenized cream contained relatively more of the lower melting-point fats still in the liquid state. A further apparent consequence of the higher degree of fat dispersion was the relatively longer time required for the homogenized cream to reach thermal equilibrium after a heating period in the calorimeter; a matter of 20 or 30 min.

Similar effects related to the degree of fat dispersion have recently been recorded by Short (18) in this laboratory, in studying density variations in enriched, ordinary and

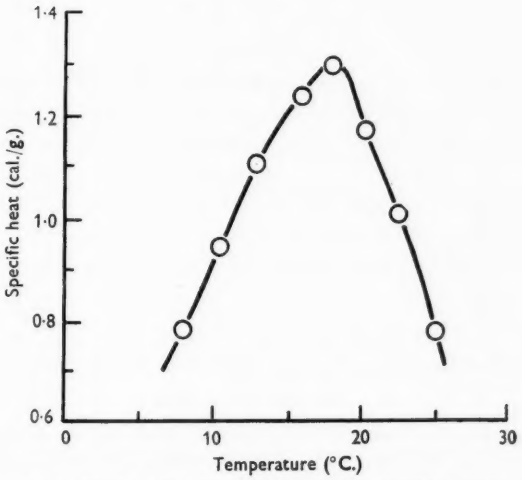

Fig. 8. Specific heat curve for the fat in homogenized cream after rapid cooling and storage at 0° C. for 16 hr.

homogenized milk. A sinker method employing Archimedes' principle was used. The milks were subjected to cooling treatments and storage at 10° C. Under these conditions, marked differences were found in the proportions of fat solidified, even up to 48 hr. after cooling.

Although not of a calorimetric nature, the following relevant experiments with a slightly modified form of Short's apparatus, enabled a useful comparison to be made of the rates of solidification of the fat in ordinary and homogenized cream (15% fat) and of butter fat in the continuous state when cooled to the same temperature. Briefly, a narrow-necked glass capsule was filled with the appropriate cream, on whose surface a containing layer of liquid of suitable density (benzyl cyanide) was introduced, immiscible with the cream and with water. After warming to 45° C., the capsule, suspended by a fine wire from one arm of a balance, was completely immersed, open-end uppermost, in a distilled water-bath at 5° C. Its apparent weight was recorded at intervals. As the fat in the cream solidified, so it contracted and the apparent weight of the capsule increased. In the case of the butter fat (moisture free) whose density was less than that of water, the method of Williams (22) was followed, in which the capsule containing the melted fat was immersed

in the inverted position with no separating liquid. Care was taken to ensure that no air bubbles were trapped within, or at the surface, of the fat.

From the graphs of apparent weight against time after cooling (Fig. 9), the progressive solidification of the fat in the various samples can be traced as in the parallel calorimetric curve of Fig. 1. In the case of the ordinary cream, a constant apparent weight, indicating completion of solidification, was reached in about 5 hr. after cooling. This is in agreement with the calorimetric result. On the other hand, the apparent weight of the homogenized cream after this time, was lower than that of the ordinary cream (same initial mass) and still rising, showing that solidification was incomplete. The butterfat solidified in approximately 2 hr. The fluctuation in the early part of this curve is undoubtedly due to the release of the major part of the heat of solidification.

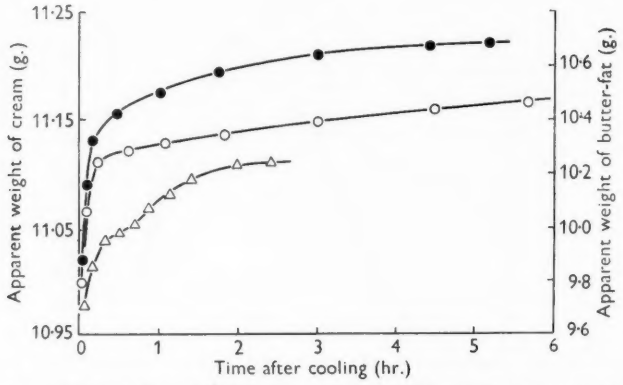

Fig. 9. Curves of apparent weight against time after cooling, illustrating the progress of crystallization of butter-fat, and the fat in ordinary and homogenized cream cooled to 5° C. ●—●, ordinary cream; ○—○, homogenized cream; △—△, butter-fat.

In connexion with the above phenomena, Rishoi & Sharp(14) considered that once seeding was established in fat in the continuous form, crystallization spread readily throughout the mass, whereas with globular fat, seeding effects were localized to individual globules, thus retarding the overall crystallization. Whilst this is very likely to be true, additional factors may be responsible for impeding crystallization. For example, the materials adsorbed on the globule surfaces may, through molecular linkages, be exerting a controlling influence, which becomes more predominant as the globule size decreases, that is, as the surface area/volume ratio increases.

CONCLUSIONS

The experiments described clearly indicate that the form of the specific heat/temperature relationship for milk fat is largely dependent upon the form of thermal pretreatment to which it is subjected. Coupled with the effects of such complicating factors as the degree of dispersion of the fat, the complexity of the melting and solidification processes of the fat in cream and milk is apparent.

Factors affecting the average globule size and/or chemical composition of the fat in cream, such as breed of cow, type of feed, season, etc., are to be borne in mind. Rishoi &

Sharp (14) purport to have detected differences in the thermal behaviour of fat from cream produced at different times of the year, and attribute this to effects of season or feed of the cow. Generally speaking, the influence of variations of this kind are likely to be small, and deviations from the typical thermal data presented here, relatively insignificant.

The investigation has been carried out under more or less ideal experimental conditions with comparatively small samples, and in these circumstances there is little or no uncertainty in the efficiency of the thermal processing methods employed. Imprecise thermal control in processing could lead to variations in the fat behaviour. This might arise in commercial practice when treating large quantities of cream, particularly if this were of a high fat content. Thermal conductivity and compound factors like thermal diffusivity, together with the presence, or otherwise, of agitation of the cream, could be relevant considerations.

SUMMARY

Specific heat data have been obtained for separated milk, whole milk, cream (15% fat content) and the fat in cream, under certain well-defined experimental conditions. With standardized techniques of sample preparation, the results were repeatable within the errors of the experimental methods employed.

An examination of the effects on the specific heat curves of milk fat of different modes of thermal treatment has provided information on the melting and solidification behaviour of the fat. The degree of dispersion of the fat in cream has been shown to be an important consideration.

My thanks are due to Miss H. R. Chapman of the Institute dairy, for supplying milk and cream samples, and to Mr J. H. Prentice and other members of the Physics department for useful discussion.

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654. A RAPID METHOD FOR ESTIMATING THE SIZE DISTRIBUTION OF FAT GLOBULES IN CREAM

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(With 4 Figures)

Equipment for treating cream should not cause clumping of fat globules and oiling-off of the fat, nor should it cause splitting up of globules into smaller globules which would be lost in the buttermilk when the cream is churned. Such effects of the equipment on the state of dispersion of the fat may be studied by comparing the size distribution of the fat globules in the cream before and after treatment.

The size distribution may be estimated by microscopic examination of the cream (1). This method, however, is laborious since it involves measuring some two or three thousand globules in each sample and then making a number of calculations to convert the distribution results from numbers of globules to percentages of fat. The microscopic method is also very subject to sampling errors, particularly in the estimation of the small number of large globules, which carry a considerable proportion of the fat in the sample.

For many purposes it is not necessary to know in detail the size distribution of the globules in the sample. It would be sufficient if the proportion of fat present as small globules (say under 2μ) and as large globules (say over 10μ) could be estimated. It seemed possible that this information could be obtained by mechanical separation of the fat globules into two or more different size groups and determination of the fat content of the respective fractions.

This principle has been used by McDowall (2) to measure variation in the amount of breaking-up of fat globules in cream with different types of treatment in a Vacreator. Cream coming from the Vacreator was passed at a temperature of 55° C. through a centrifugal separator of the type used for producing 80% cream. The fat content of the skim milk fraction was taken as a measure of the fat present as small globules in the cream. This method provided some useful results, but had the disadvantage that a considerable volume of cream was required for each run and that there was some difficulty in ensuring that the conditions of separation remained the same for each lot of cream compared. It would not have been possible to draw samples from different parts of the Vacreator without the introduction of additional equipment which might itself have affected the fat globules in the cream.

Separation of a serum by centrifuging cream in a laboratory centrifuge appeared to be a more convenient method. To ensure that the fat was present as single globules and not as clusters, it would be necessary to keep the temperature during this operation high enough to prevent clustering. It has been suggested by Whittlestone (3,4) that clusters of globules may be present in milk at temperatures as high as blood heat. A higher temperature than this would, therefore, be necessary during centrifuging.

For estimation of the larger globules and oiled-off fat, a gravity creaming technique seemed possible. Here again the temperature would have to be kept high enough to ensure the absence of clusters.

ESTIMATION OF PROPORTION OF FAT PRESENT AS SMALL GLOBULES

Methods

The cream samples under investigation were heated to 60° C. in centrifuge tubes and then spun in a laboratory centrifuge fitted with an electric heater to maintain the temperature. In all the experiments the centrifuge was operated at 3000 r.p.m., giving a relative centrifugal force of 2000 ***g***.

The fatty layer above the serum was removed as completely as possible by suction, and a sample was then taken with a pipette from the bottom of the tube. Determinations by the Mojonnier method were made of fat in the uncentrifuged sample and in the serum. The proportion of the fat remaining in the serum was then calculated.

Results

When cream of 35–40 % fat was centrifuged the separation of fatty and watery layers was very incomplete, but if the cream was first diluted with 2 or 3 times its volume of water the two layers separated sharply.

The proportion of fat remaining in the serum was found to depend not only on the speed and time of centrifuging but also on the depth of liquid in the tube and on the concentration of fat in the diluted cream. Microscopic examination of serum samples showed that there was a less complete removal of large globules in a full 100 ml. tube than in a full 50 ml. tube centrifuged under the same conditions. All subsequent work was done with 50 ml. centrifuge tubes filled as full as possible consistent with balancing. Two tubes were usually set up on each sample and the serum portions were combined.

Variation in fat content of serum with sampling level. Cream (47 % fat) diluted to 12 % fat was centrifuged for 15 min. Three successive 10 ml. portions of serum were withdrawn from the bottom of the tube, using an Andreasen pipette (see later, Fig. 3) to avoid mixing the serum. The results for the three successive samples were: fat content of serum (%)—0·255, 0·263, 0·278; serum fat/total fat (%)—2·14, 2·21, 2·33.

It was concluded that a 20 ml. serum sample could be taken from each 50 ml. tube without including any upper layers of high fat content, and that small variations in the volume of sample taken would not affect the fat content.

Effect of fat content of diluted cream and of time of centrifuging. The results shown in Fig. 1 indicate that, for a given time of centrifuging, the proportion of the fat left in the serum depends on the fat content of the diluted cream. Only a small part of this variation can be explained by changes in the viscosity of the cream serum with dilution. Apparently it is principally due to a hindrance effect between the fat globules when they are closely packed. The change with dilution is negligible at lower fat contents (below 10 %) and with times of centrifuging of 20 or 30 min. As the fat content of the diluted cream is increased, the change becomes more and more marked. This hindrance effect will explain the very incomplete separation obtained with undiluted cream.

It appears also from the curves in Fig. 1 that where the diluted cream is centrifuged

for a sufficient time the proportion of fat left in the serum reaches a minimum and that more prolonged centrifuging will produce very little further decrease in fat content. This horizontal part of the curve is a convenient range within which to operate when using this method to determine the proportion of small globules in cream. Where the cream is diluted to about 10% fat and centrifuged for some 30 min. the fat content of the serum is not affected by small variations in either fat content of diluted cream or in time of centrifuging. Dilution to a fat content much below 10% would not be advisable since the fat content of the serum would be too low for accurate estimation.

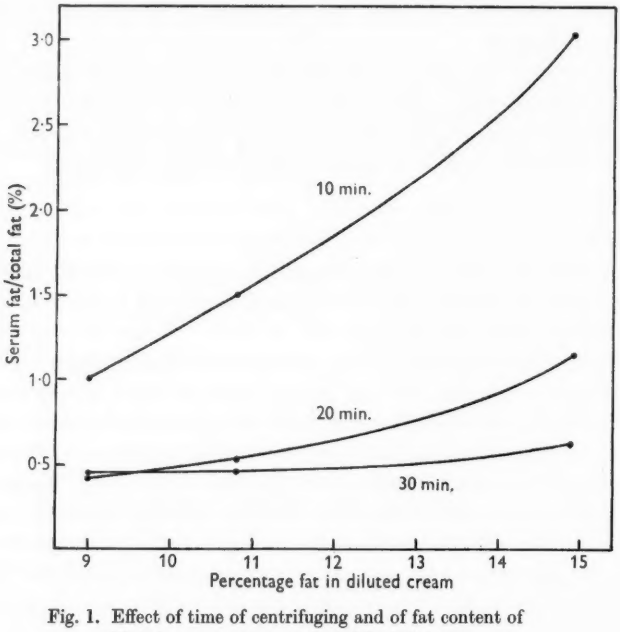

Fig. 1. Effect of time of centrifuging and of fat content of diluted cream on proportion of fat left in cream.

Size of fat globules retained in the serum. Microscopic counts were made of the larger fat globules in the serum samples from the above experiment. For this purpose the serum samples were diluted with an equal volume of glycerol and each was examined in a cell 10μ deep so that the depth of preparation was the same for all samples. All the globules of diameter 1μ and over were measured in six fields on each sample. The results, corrected for the dilution of the samples, are given in Table 1.

The numbers of globules showed changes, with time of centrifuging and fat content of diluted cream, similar to those in the fat percentages in Fig. 1. Serum samples centrifuged to the minimum fat content contained no globules over 1·5μ diameter. All but a very small proportion of the 1·5μ globules were removed by centrifuging, and there was an appreciable reduction in the number of 1μ globules.

A complete microscopic measurement of the size distribution of fat globules by the method described in an earlier publication (1) was made on another portion of the cream used in the above experiments. The results are shown in Table 2.

The proportion of fat left in the serum when the cream diluted to 10% fat was centrifuged for 20–30 min. (0·46%) corresponds closely with the figure (0·5%) for the microscopic estimate of fat as globules 1 μ and less in diameter. It may be concluded that this is the approximate size of globules estimated by the centrifugal method under the conditions specified.

Table 1. *Numerical distribution of fat globules in centrifugal serum from diluted cream (corrected for dilution)*

Fat in diluted cream (%)	Time centrifuged (min.)	Globule diameter (μ) 1	1·5	2	3	4
14·9	10	*	465	111	6	3
	20	891	57	3	—	—
	30	420	12	—	—	—
10·9	10	1120	188	24	—	—
	20	620	8	—	—	—
	30	516	20	—	—	—
9·0	10	2060	110	5	—	—
	20	420	10	—	—	—
	30	290	5	—	—	—

* Not counted.

Table 2. *Size distribution of fat globules in cream*

(Expressed as percentage of total fat.)

Globule diameter (μ)	0–1	1·5	2–3	4–6	7–10	>10
Fat in size class (%)	0·5	1·5	26	52	19	1

Relation between size distribution of globules in cream and serum. As shown by the results in Table 1, the serums contain a range of sizes of fat globules. This size distribution may be related to that in the original cream if the diameter of the largest globules in the serum is known. The method may be illustrated as follows:

Fig. 2*a* represents a centrifuge tube and its contents at the end of centrifuging. (For convenience the tube is shown as flat-bottomed.) The portion of serum to be taken as a sample is that from A up to the level D. It is assumed that fat globules of each size rise at a rate proportional to the square of their radius (Stokes's Law) and that the centrifuging has been stopped when globules 2 μ in diameter, which were initially at the bottom of the tube, have risen to the level D. The serum sample will therefore contain no globules of diameter 2 μ or over. Globules 1 μ in diameter will have risen only one-quarter of the distance AD, i.e. to level B. The sample will therefore contain three-quarters of the concentration of 1 μ globules that was present in the cream before centrifuging. Similarly, the concentration of globules 1·5 μ in diameter will be $1-\frac{(1{\cdot}5)^2}{(2)^2}=\frac{7}{16}$ or 43·75% of the original.

An illustration of the globule sizes to be expected on this basis is given in Fig. 2*b*. The upper curve is part of the size-distribution curve determined microscopically for the fat globules in a particular cream sample. The lower curve is the distribution curve for the serum calculated on the assumption that the centrifuging was just sufficient to remove 1·5 μ globules from the serum. (This corresponds with results in Table 1 and other observations for cream diluted to 10% fat and centrifuged for 30 min.) The area under

the lower curve represents the fat content of the serum sample (as percentage of the total fat). Since the two areas shown cross-hatched on the diagram are equal, the total area under the lower curve corresponds with the area under the upper curve between 0 and $1{\cdot}2\mu$, i.e. fat as globules over $1{\cdot}2\mu$ included in the serum is equal to fat as globules under $1{\cdot}2\mu$ in the cream and not included in the serum.

This upper limit will vary to some extent with changes in the shape of the size distribution curve for the cream, but it may be concluded that in general the proportion of fat in serum obtained under the conditions specified will correspond approximately with the proportion of fat present as globules up to and including 1μ in the cream. The method should, therefore, give a useful indication of the proportion of small globules in the cream.

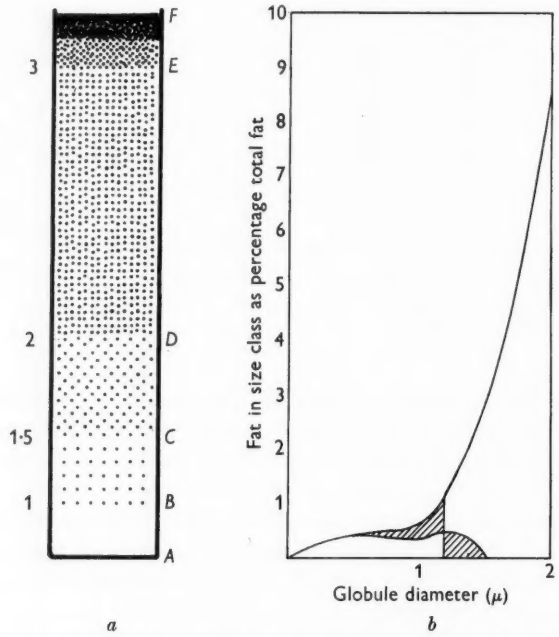

Fig. 2*a*. Separation of fat globules in centrifuging. Fig. 2*b*. Size distribution of fat globules in original cream and in centrifuge serum.

ESTIMATION OF PROPORTION OF FAT PRESENT AS LARGE GLOBULES

Methods

Cream was diluted and a portion of the fat was allowed to rise by gravity creaming, the temperature being maintained at 60° C. to eliminate clusters.

To permit sampling at a fixed depth there was used a modification of the Andreasen particle-size apparatus(5,6). This apparatus consists of a tall glass jar to which is fitted, by a B29 ground-glass joint, a pipette with a two-way tap (see Fig. 3A). The jar is graduated in centimetres and the tip of the pipette is level with the lowest graduation (zero). For particle-size measurements the jar is filled to the 20 cm. mark with a suspension of the particles. Samples are taken at regular intervals by means of the pipette.

The decrease noted in the solids content of successive samples indicates the quantity of the suspended material which has fallen 20 cm. in the given time.

It was necessary to modify the apparatus to allow measurement of the amount of fat which would rise 1 cm. in a given period. For the earlier experiments, the jar was filled with mercury to within 1 cm. of the tip of the pipette. For later work, the jar was replaced by a 250 ml. conical flask with a B29 socket. The pipette was cut off and the tip

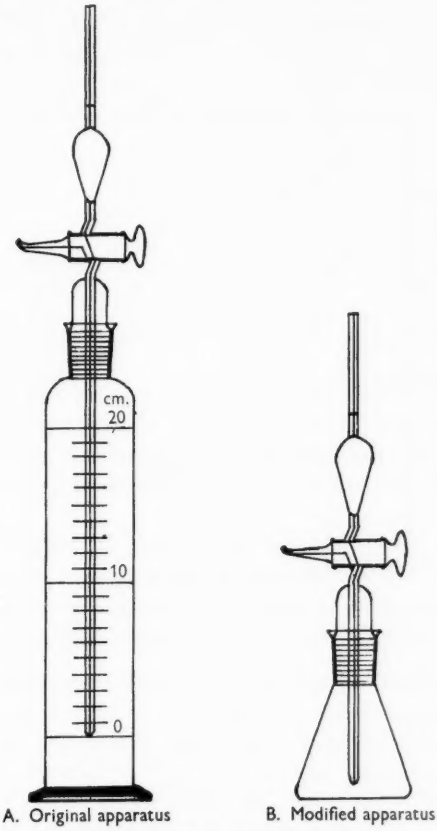

Fig. 3. Andreasen particle-size apparatus.

ground so as to terminate 1 cm. from the bottom of the flask. To facilitate this operation, a flat metal cylinder rather less than 1 cm. high was placed in the flask. The clearance between the tip of the pipette and the top of the cylinder was measured with a vernier microscope and the grinding was continued until the clearance plus the height of the cylinder was 1 cm.

The jar or flask was half-filled with diluted cream at 60° C. and the apparatus was placed in a thermostat bath. Samples were withdrawn at the beginning and end of a measured period and the fat content of each was determined by the Mojonnier method. The difference in fat content of the two samples expressed as a percentage of the total fat content (given by the first sample) indicated the percentage of the fat which had risen 1 cm.

Results

Effect of fat content of diluted cream and of time of standing. It was found (see Fig. 4) that the proportion of fat which rose 1 cm. in a given time was reduced as the fat content of the diluted cream was increased. In this respect the results were similar to those on centrifuge serum (Fig. 1), but in the gravity creaming trials there was, over the range of fat contents investigated, no tendency for the change with fat content to become less at low fat contents. To secure comparable results on different samples, all must therefore be diluted accurately to the same fat content. A dilution to 10% fat, as with the centrifugal determination, was found convenient.

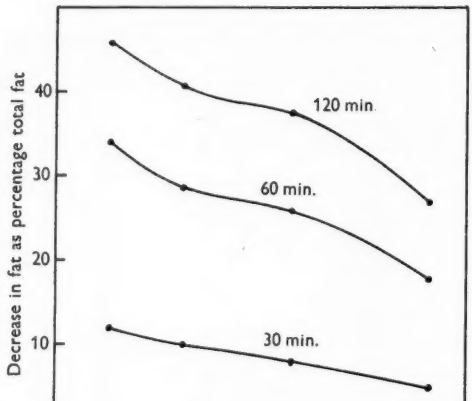

Fig. 4. Effect of time of standing and of fat content of diluted cream on proportional decrease in fat content at 1 cm. level in Andreasen apparatus.

Size distribution of globules in samples from Andreasen apparatus. Creams diluted to 10 and 5% fat were placed in the Andreasen apparatus and sampled at 0, 30, 60 and 120 min. Portions of these samples were diluted to the same fat content, mixed with gelatin solution and examined under the microscope in cells 10μ deep. Globules of diameter 5μ and over were counted in nine fields on each sample. The results are given in Table 3.

With creams diluted to 10% fat, there was a significant reduction in numbers of globules over 8μ diameter in samples taken after 30 and 60 min. and of globules over 5μ at 120 min. With cream diluted to 5% fat, the corresponding reduction in numbers occurred with globules over 9, 7 and 5μ diameter respectively at sampling times of 30, 60 and 120 min.

With uniform conditions for rise of globules during standing and for sampling at an exact level without disturbing the surrounding liquid, a sample taken after a period of standing should contain no globules above a certain size and the concentrations of globules of less than this critical diameter should be the same as in the initial sample. The results in Table 3 do not show such a sharp cut-off but a more gradual reduction in numbers of globules. Some allowance must be made for errors in the microscopic technique—

sampling variations and variations from the nominal 10μ in the depth of preparations counted. It is probable, however, that the cause of the lack of sharpness is in the creaming apparatus. The sample taken with the pipette will come from a range of levels above and below the 1 cm. level. Any movement of liquid due to convection currents or other causes will make the boundary between layers of globules less sharp.

Table 3. *Numerical distribution of fat globules in diluted cream after various periods of standing*

Globule diameter (μ)	10%				5%			
	Initial	30 min.	60 min.	120 min.	Initial	30 min.	60 min.	120 min.
	(Totals for 9 fields 10μ deep)							
5	57	56	60	46	51	49	33	33
6	29	21	40	14	34	21	10	7
7	20	22	12	4	27	19	12	2
8	15	12	11	3	13	9	2	1
9	13	3	3	—	5	6	—	—
10	6	3	1	—	8	3	—	—
11	4	2	1	—	4	1	—	—
12	2	—	—	—	1	—	—	—
13	—	—	—	—	—	1	—	—
14	2	—	—	—	—	—	—	—
15	1	—	—	—	1	—	—	—

Other similar experiments made with cream diluted to 10% fat showed that irregularities in number of globules were more pronounced with a 30 min. standing time than with longer periods. It seemed likely that steady conditions were not reached till some minutes after the start of the time period. The longer period of 60 min. was therefore to be preferred.

Estimations made on cream diluted to 10% and allowed to stand for 60 min. should indicate as 'large globules' those approximately 7 or 8μ and over in diameter.

DETAILED METHOD FOR ESTIMATION OF FAT AS LARGE AND SMALL GLOBULES

On the basis of the experiments described above, the following method has been found suitable:

Determine the fat content of each sample by some rapid method, e.g. Gerber test. Dilute each sample with water to 10% fat content.

(If only small globules are to be estimated and the fat content of the sample is known with sufficient accuracy to ensure that the dilution is between 9 and 11% fat content, the preliminary fat determination may be omitted.)

Estimation of fat present as large globules. Heat the diluted sample to 60° C. and place 200 ml. in a modified Andreasen apparatus (Fig. 3B). Mix thoroughly by inverting the flask, then remove two 10 ml. portions with the Andreasen pipette for estimation of fat by a gravimetric method (e.g. Rose-Gottlieb or Mojonnier). (Fat % *a*.)

Place the apparatus in a thermostatic bath at 60° C. Allow 15 min. for the temperature of the contents to become constant, then mix the sample by inverting the apparatus. Replace the apparatus in the thermostat and note the time. After 60 min. take two more 10 ml. samples for fat estimation as before. (Fat % *b*.) (The second 10 ml. sample always has a slightly higher fat content than the first. The mean of the two determinations is taken.)

Estimation of fat present as small globules. Fill two 50 ml. centrifuge tubes from each

sample of 10% diluted cream. Heat to 60° C. and spin in a heated centrifuge at 3000 r.p.m. for 30 min. Samples to be compared should all be centrifuged in one batch.

Draw off the fat and cream layer from each centrifuge tube as completely as possible and then pipette 20 ml. of serum from the bottom of each tube. Combine the two 20 ml. portions of serum and make duplicate or triplicate gravimetric fat estimations on each serum sample. (Fat % *c*.)

Calculation of results:

$$\text{Percentage of fat present as large globules} = \{(a-b)/a\} \times 100.$$
$$\text{Percentage of fat present as small globules} = (c/a) \times 100.$$

It is not considered necessary to correct for the error in c/a resulting from measuring diluted cream at 60° C. instead of 20° C., since the error is small compared with that in the determination of fat in the serum.

Typical results. As an illustration of the results obtained by this method, the following data are given for a cream before and after treatment in a Vacreator:

	Raw	Treated
Fat as large globules (%)	13	42
Fat as small globules (%)	1·2	1·7

The results of other estimations by this method will be given in a subsequent paper.

SUMMARY

The proportion of fat present in cream as small globules (approximately 1 μ and under) is estimated from the proportion of the total fat which remains in the serum when a sample of the cream, diluted to 10% fat, is centrifuged at 60° C. for 30 min. at 3000 r.p.m.

The fat present as large globules (7 or 8 μ and over) is estimated by finding the proportion of the fat which rises 1 cm. in 60 min. when the cream, diluted to 10% fat, is held at 60° C. in a modification of the Andreasen particle-size apparatus in which the jar is replaced by a conical flask and the tip of the sampling pipette is held 1 cm. from the flat bottom of the flask.

The thanks of the author are due to Dr F. H. McDowall for helpful advice and criticism and to Miss R. P. Johnson for assistance with the experimental work.

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655. THE EFFECT OF pH ON THE EXTENT OF SPLITTING OR CLUMPING OF FAT GLOBULES CAUSED BY AGITATION OF HOT CREAM

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(With 1 Figure)

High fat content in New Zealand buttermilk powder has been found to occur principally in samples of low acidity (Beange (1)). This indication that over-neutralization of cream could cause increased fat losses in the buttermilk suggested that there must be a relationship between the pH of the cream and the susceptibility of the fat globules to subdivision during the pasteurization and deodorization treatment. The present paper gives the results of an investigation of the effect of pH on changes in the size distribution of fat globules in cream brought about either by Vacreator treatment or by mechanical agitation such as could occur in other pasteurization or deodorization equipment.

METHODS

Cream to be treated in the Vacreator was bulked in a weighing vat and successive portions were run into a neutralizing vat, brought to the desired pH by addition of N/2 sodium hydroxide or lactic acid, and then passed through a tandem Vacreator. Samples were taken from a sampling cock close to the extractor pump. They were cooled quickly to 20–25° C. and examined with a minimum of delay.

Other cream treatments were carried out in the laboratory by methods described in the appropriate sections.

Estimations of size distribution of fat globules were made either by the microscopic method (2) or by a more rapid method involving separation of small and large globules by centrifuging and gravity creaming respectively (3). In each case the fat present as globules of a particular size class was expressed as a percentage of the total fat.

RESULTS

Vacreator experiments

Size distributions by microscopic method

The results of three experiments in which the samples were examined by the microscopic method are set out in Table 1. In the first two experiments, samples of cream treated at high pH (7·45 and 7·3 respectively) contained a much higher proportion of fat as small globules ($< 2\mu$) than did samples treated at the lower pH values (6·8 and 6·75).

The third experiment included also an investigation of the effect of intensity of treatment in the Vacreator.* The two creams treated at low intensity contained a

* Figures given for intensity of treatment represent the weight of steam used for deodorizing purposes per gallon of cream and do not include steam used for heating the cream to the temperature at which deodorization is carried out. Intensity is estimated from the rise in temperature of the condenser water and the rates of flow of condenser water and cream.

proportion of fat as small globules which did not differ significantly from that in the cream before treatment. There was, however, a considerable increase in the proportion of fat present as large globules ($>10\mu$), showing that the treatment, though mild, had caused clumping of globules. In the creams treated as high intensity, the low pH cream (pH 6·55) contained slightly less fat as small globules but considerably more as large globules than the original cream. The high pH cream (pH 7·1) contained considerably more fat as small globules than the original cream and also showed an increase in fat as large globules. The proportion of fat as large globules was, however, much less in the cream at pH 7·1 than in the cream at pH 6·55 treated at the same intensity.

Table 1. *Effect of* pH *of cream on size distribution of fat globules in Vacreator-treated cream*

(Microscopic method.)

Date	Fat in raw cream (%)	pH of raw cream	Intensity of treatment (lb. steam per gal. cream)	Fat in globule size class as percentage total fat				
				$<2\mu$	$2–3\mu$	$4–6\mu$	$7–10\mu$	$>10\mu$
26. xi. 52	37	6·8	3·4	2·2	14	48	31	5
		7·45	3·6	4·4	19	44	32	1
10. xii. 52	38	6·75	4·3	2·1	11	38	34	14
		7·3	4·0	3·5	14	40	26	17
30. iii. 53	40	Untreated		1·9	17	55	24	2
		6·55	2·0	1·8	10	30	29	29
		7·1	2·0	2·0	10	31	29	29
		6·55	5·2	1·6	9	29	38	22
		7·1	5·3	3·0	13	35	35	14

Size distribution by rapid method

The use of the more rapid method of assessing the proportion of fat present as small and as large globules made it possible to examine in more detail the changes occurring with alteration of pH. The results of two further trials are set out in Table 2, and those of a third, which appears to give a good picture of the whole phenomenon, are shown in

Table 2. *Effect of* pH *of cream on size distribution of fat globules in Vacreator-treated cream*

(Centrifugal and creaming method.)

Date	Fat in raw cream (%)	pH of raw cream	Intensity of treatment (lb. steam per gal. cream)	Fat in globule size class as percentage total fat	
				Small globules	Large globules
20. ii. 56	41	Untreated		1·25	13
		6·0	3·4	1·6	76
		6·5	3·4	1·5	51
		7·0	3·4	1·65	43
		7·4	3·0	—	29
		7·95	3·4	—	29
12. iii. 56	36·5	Untreated		0·55	—
		6·0	6·1	1·7	—
		6·6	5·8	2·0	—
		6·95	5·9	2·1	—
		7·5	6·0	2·4	—
		8·2	6·2	3·7	—

graphical form in Fig. 1. As the pH of cream increased from 6 to 7 there was a gradual rise in the proportion of fat present as small globules (1 μ and under) in the treated cream. From pH 7 to 8 the rise was much steeper. The clumping of globules (indicated by increase in the proportion of fat present as large globules, over 7 or 8 μ) was at a minimum in cream treated at pH 8 and increased rapidly with lowering of the pH.

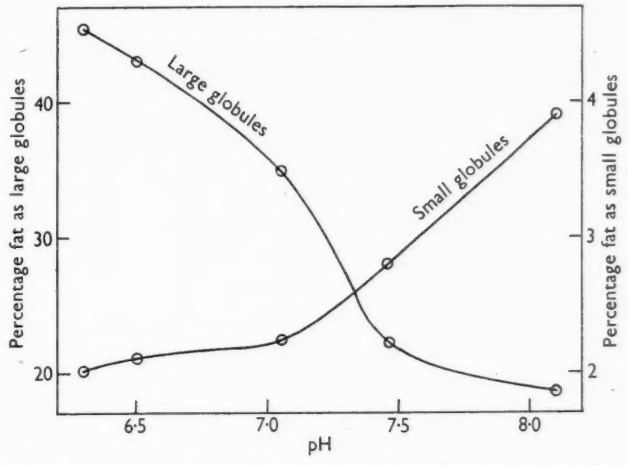

Fig. 1. Effect of pH of cream on proportion of fat present as large and as small globules in Vacreator-treated cream. Intensity of treatment 6·0. Values for untreated cream: percentage fat as small globules 0·8; percentage fat as large globules 10.

The results given in Fig. 1 show the effect of a high intensity treatment, in which extensive splitting up of fat globules can occur. Other experiments have indicated that as the intensity is reduced the curve for proportion of fat present as small globules becomes flatter (i.e. the effect of pH is less apparent), but that for large globules is very little changed even at a low intensity.

Mechanical agitation of cream

Experiments, to be described elsewhere, have indicated that breaking up of fat globules in the Vacreator occurs only in parts of the equipment where a mixture of steam and cream attains a high velocity and that the action is due to a violent agitation of the cream in the presence of a vapour phase. The effect of various methods of agitating cream was investigated on the laboratory scale in order to find if a similar behaviour would take place under these conditions.

Mechanical stirring of hot cream producing a moderate degree of agitation caused clumping, but very little splitting of the globules. A more vigorous agitation, e.g. treatment in a Waring Blendor, brought about changes in the globules similar to those taking place in a Vacreator.

Portions (100 ml.) of one lot of cream were adjusted to various pH values and were beaten for 60 sec. at 60° C. in a Blendor. Typical results obtained on Blendor-treated creams (Table 3) showed the same variation with pH as did Vacreator-treated creams.

It was noted that the stability of creams treated in the Blendor varied markedly with the pH at which the cream was treated. Creams agitated at a pH near 6·5 tended to become partially converted to butter on standing for a few hours, and if heated after being held overnight at 10° C. they yielded a large amount of oiled-off fat. Samples

Table 3. *Effect of* pH *of cream on size distribution of fat globules in cream agitated in Blendor*

Date	Fat in cream (%)	pH of cream	Fat in globule size class as percentage total fat: Small globules	Large globules
3. x. 55	Untreated		0·6	24
	40	5·65	2·0	50
	40	6·0	2·0	34
	40	6·5	1·8	34
	40	7·25	2·2	28
	40	7·95	3·0	11

agitated at pH 7·5–8 showed little or no tendency to form buttery masses or 'cream plug' on standing, and gave very little oiled-off fat when reheated. These observations agreed with the estimates of the proportion of fat as clumped or large globules in the samples.

Aeration without mechanical agitation

To determine the effect of aeration alone on cream, experiments were conducted as follows:

Cream (100 or 120 ml.) was heated to 60° C. and placed in a beaker in a water-bath held at 60° C. Air, preheated to the temperature of the bath, was blown through the cream, using a sintered glass distributor from a gas wash-bottle to produce fine bubbles. The rate of airflow was measured by a flowmeter.

A copious foam was formed on the cream. It was prevented from overflowing the beaker by use of an air jet to break the upper layers of foam.

In a preliminary experiment 100 ml. portions of 40 % cream were aerated at 400 ml./min. for 10 min. The proportion of fat present as large globules was found to be increased from 7·9 % in the original cream to 10·4 % by aeration at pH 7·9 and to 19·8 % by aeration at pH 6·2. The aeration at low pH thus caused a much greater amount of clumping.

The results of a more detailed experiment in which 120 ml. portions of freshly separated 40 % cream were aerated at 1000 ml./min. for 10 min. are set out in Table 4.

Samples aerated at a pH above 7 showed a definite increase in fat as small globules, but only a small increase in fat as large globules. Those aerated at a pH below 7 showed a decrease in the fat present as small globules and a considerable increase in that as large globules. The trends were not very regular and may have been influenced by small differences in the extent of aeration. The results are sufficiently clear-cut, however, to show that pH has a definite effect on the extent of splitting or clumping of globules produced by aeration without mechanical agitation.

Homogenization of cream

In all the experiments described above, bubbles of air or steam in the liquid cream probably played a part in the clumping or splitting of the globules. In order to determine whether pH could affect the splitting of globules in the absence of an air or vapour phase, an experiment was made on the homogenization of cream at various pH values.

Table 4. *Effect of* pH *of cream on size distribution of fat globules in aerated cream*

pH of cream	Fat in globule size class as percentage total fat: Small globules	Large globules
Untreated	1·1	9·5
6·0	0·9	16
6·45	0·9	17
7·0	1·3	18
7·5	1·4	10
8·2	1·3	12

A quantity of cream was diluted with skim milk to bring the fat content to about 20% and was divided into three portions (1·5 l. each) which were adjusted to approximately pH 6, 7 and 8 respectively by addition of N lactic acid or sodium hydroxide. The creams were held overnight at 10° C. and were then heated to 60° C. and passed through a single-stage homogenizer at a pressure of 2500 lb./in.^{2} The fat content, pH and viscosity of the homogenized creams were determined. The pH values of the creams were then adjusted to bring all the samples to the same pH value and the viscosities were again determined. The creams were also examined under the microscope.

The cream homogenized at pH 6 showed to a pronounced extent the increase in body normally found when cream is single-stage homogenized. That treated at pH 7 showed little or no increase in body, while the cream homogenized at pH 8 was even more fluid. These observations were confirmed by determinations of the viscosities of the creams at 40° C. in a Hoppler viscosimeter (see Table 5).

Table 5. *Effect of* pH *of cream on viscosity of homogenized cream*

Values after homogenization: Fat (%)	pH	Viscosity (c.p.)	Values after readjustment of pH: pH	Viscosity (c.p.)
21·0	6·2	82	6·9	27
21·3	6·9	4·3	6·9	4·2
20·8	7·6	3·8	6·9	2·9

Under the microscope the cream homogenized at pH 6 showed to a marked degree the clumping of small globules which commonly occurs in single-stage homogenized cream—the globules formed a continuous flocculent mass. Cream homogenized at pH 7 had only small masses of flocculated globules, while that homogenized at pH 8 contained only separate globules and thus resembled two-stage homogenized cream.

DISCUSSION

It has been established that when hot cream is agitated in contact with steam or air so as to cause some clumping or splitting of the globules, the effect of the treatment on the state of fat dispersion in the cream depends on the pH of the cream. At a pH near 8, splitting is at a maximum and clumping at a minimum. At a pH near 6 the reverse is the case.

Previous work on the behaviour of milk fat during agitation of milk and cream was reviewed by King in 1951 (4). From the evidence available, including his own microscopic observations of the surface of milk by reflected light, King concluded that the important factor is the incorporation of bubbles of air into the liquid by the agitation, the mechanism being as follows: fat globules, the surface of which has become hydrophobic, collect at the air-plasma interface and, when the bubbles burst, the globules are forced together into clumps bound together by butter oil. At temperatures above the melting-point of the fat, the globules at the interface spread out to form lenses which, on the bursting of the bubbles, are shattered into numerous minute droplets of fat and distributed through the plasma. With milk at lower temperatures clumping predominates, while above the melting-point of the fat the principal action is splitting of the globules.

The results of the present investigation show that King's conclusions do not all apply to cream. Here, even at temperatures well above the melting-point of the fat, the predominant effect of agitation is clumping. The results of the agitation of hot cream were more accurately described by Samuelsson (5), whose conclusions were summarized in another review by King (6) as follows: 'At temperatures above the melting-point of the fat a slight mechanical treatment causes coalescence of the fat globules, in other words, breaking of the emulsion, while on violent churning a splitting of globules predominates, especially in the case of a cream of lower fat content.' The experience of the present author supports the view of Samuelsson that as the fat concentration is raised clumping is increased and splitting reduced, i.e. closer packing of fat globules favours aggregation.

There is a considerable amount of information on the effect of pH on the churning or agitation of cream at temperatures below the melting-point of the fat. It is general experience that acid creams churn more readily than sweet creams. According to Guthrie & Sharp (7) churning time is at a minimum at pH 4·2 and a maximum at pH 10. It appeared to these workers that change of churning time was associated with dispersion or hydration of casein at higher pH values and precipitation or dehydration at lower values approaching the isoelectric point.

If we accept the view of Mohr & Mohr (8) that clumping is the first step towards churning, the observations on rates of churning indicate that, at temperatures at which the fat is partly solidified, lower pH values favour clumping. It has also been shown by Nugent (9), in studies of the behaviour of fat globules at an oil-cream interface (presumably at room temperature), that above pH 6 the resistance of fat globules towards disruption on contact with the interface increases rapidly with pH reaching a high value at pH 8. Nugent attributed these results to a variation with pH in the hydration of the protein adsorbed on the globule surface.

A very different indication was found by King (10) in an investigation of the number of globules brought to a milk surface at 16–17° C. by touching it with a platinum ring. In the pH range 4·8–7·8 there was a minimum at pH 5·3, showing that the tendency for globules to become hydrophobic was least at this pH and increased as the pH was raised.

Reports of investigations of the effect of pH on the stability of fat globules in cream agitated at temperatures above the melting-point of fat appear to be lacking in the literature. It seems from the results given in the present paper that at higher temperatures the stability of the membrane is reduced as the pH is lowered, just as it is in churning at lower temperatures, i.e. as pH is lowered the membrane becomes less stable and the tendency for clumping is increased. The increased amount of splitting of globules at higher pH values may be explained in the same way. Where the membrane material is sufficiently stable, fat surfaces exposed by subdivision of globules will rapidly be covered by a new membrane and the small globules will be prevented from re-uniting. At lower pH values, subdivided globules are not immediately protected and may clump to form globules larger than the original ones.

In all the agitation experiments reported in this paper, with the exception of homogenization, either air or a vapour phase was present, and collection of globules at the interface as suggested by King (10) probably played an important part in the process. It is possible that the pH of the cream may have affected the numbers of globules picked up by the bubbles. Differences in this direction would also point to a difference in the nature of the globule surface which must change from hydrophilic to hydrophobic to permit the globules to become attached to the interface.

The experiment on homogenization of cream also indicated a difference in the properties of the globule membranes with change in pH. The flocculation of globules occurring in homogenized cream is commonly described as 'clumping'. This term is not strictly correct if, as in the rest of this paper, 'clumping' is taken to mean incipient coalescence. The 'clumped' globules in homogenized cream do not coalesce even when the cream is cooled and reheated. The flocculation also differs from clustering since the aggregates of globules do not disperse when the cream is heated.

It appears that in cream homogenized at a pH over 7, the globules are covered with an adsorbed layer which acts just as well as the original membrane in preserving the individuality of the globules. At lower pH values a less regular adsorption takes place and, although the globules are prevented from coalescing, they are not able to remain separate but are joined by the adsorbed material.

The observations reported in the present paper should have some useful practical applications. In the treatment of cream for buttermaking by means of equipment which is liable to cause splitting of globules, the cream should not be neutralized to a pH over 7 if an increase in fat losses in churning is to be avoided. On the other hand, it is not desirable for the pH to be too low or the clumping of fat globules during the treatment may become so pronounced as to result in the presence of specks of oiled-off fat in the butter which may in consequence have a 'mealy' body.

With other methods of treatment which, because the agitation of the cream is less violent, cause very little splitting but do cause clumping of globules, a higher pH during treatment may be desirable. In such cases the cream could be neutralized to a pH between 7 and 8 before treatment and reacidified afterwards.

Applications to other operations with cream where it is desired either to produce a homogenizing effect or to secure maximum oiling-off will also suggest themselves.

SUMMARY

The effect of pH on the extent of splitting and clumping of globules in hot cream agitated by treatment in a Vacreator, by vigorous mechanical stirring or by simple aeration has been investigated. The size distribution of globules was determined in cream before and after treatment either by the microscopic method or by a rapid method involving separation of small and large globules by centrifuging and gravity creaming respectively.

It was found that at pH 8 splitting of globules was at a maximum and clumping at a minimum. As the pH of the cream to be treated was lowered towards pH 6, the amount of splitting decreased and clumping increased. The 'clumping' of globules in cream during single-stage homogenization was also increased as the pH was lowered.

Practical applications of the observations are suggested.

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656. FACTORS INFLUENCING THE VITAMIN CONTENT OF MILK FAT

II. THE INFLUENCE OF BOTANICAL COMPOSITION OF THE PASTURE

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(With 2 Figures)

INTRODUCTION

The vitamin A potency of British, European and American butters has been investigated by a number of workers. Clearly defined seasonal fluctuations have been reported and these have been shown to depend largely on variations in the carotene content of the feed available to the cows. Under New Zealand conditions, where cows are fed outdoors on pasture throughout the year, a uniformly high potency might be expected. That this was not the case was first reported by Barnicoat (1), who found marked seasonal variations in butter representative of two North Island dairying districts (Waikato and Manawatu) during the 1935–36 season. Both carotene and vitamin A were shown to be affected but variations were in the opposite direction from those reported for overseas butters, maximum potencies (42–53 i.u. vitamin A/g. fat) being recorded in late winter and spring and minimum (33–37 i.u. vitamin A/g. fat) in late summer. Subsequently these trends have been confirmed by McDowell & McDowall (2) in a more extensive investigation covering other New Zealand dairying districts and extending over several seasons, and by Farrer, Balding, Warren & Miller (3) for a number of districts in Australia where dairying conditions are comparable with those in New Zealand.

Several aspects of this problem have already been investigated. Barnicoat (1) and McDowell (4), for example, using spring- and autumn-calving cows, showed that there was no relationship between vitamin A potency and stage of lactation and concluded that variations with season were associated mainly with factors of nutritional origin.

Some of the factors which might be responsible have recently been investigated by McGillivray (5)*. In preliminary experiments it was shown that variations in the carotene content of pasture throughout the year had no effect on the seasonal change in potency of the milk fat, since at any one time the carotene content of the pasture was well above the level required for production of fat of maximum potency (6, 7). It was suggested, therefore, that the problem was one mainly of availability of carotene from the pasture. Although many factors were known to affect the availability of carotene, it appeared likely that the decreased availability from summer pasture might be associated with a low level of tocopherol in the pasture and in a later experiment McGillivray demonstrated that it was possible to raise the potency of fat to a normal spring maximum by supplementing cows grazing summer pasture with 1 g. of tocopherol per day. Furthermore, it was established that the tocopherol content of typical mixed pastures varied throughout the

* Part I of this series.

year, reaching a minimum in the late summer, and that the tocopherol content of clover was lower than that of ryegrass sampled at the same time. On the basis of these results it was further suggested that the low vitamin A potency of summer fat might be due to a high percentage of clover in the pasture since the emergence of clover as the dominant summer species (8) coincided with the production of fat of lowest potency.

It was thought desirable, therefore, that the relationship between botanical composition and vitamin A potency should be further investigated. The experiments reported in the present communication were designed to study, first, the effect of day-to-day variations in the botanical composition of pasture on the vitamin A potency of milk fat; secondly, the effect of feeding separately white clover (*Trifolium repens*, N.Z. pedigree strain), red clover (*T. pratense*), and ryegrass (*Lolium perenne*); and finally, since differences were found between the two clovers, the effect of oral dosing with thiocyanate, a goitrogenic detoxication product of the cyanogenic glucoside present in white clover but absent from red. Although the experiments were carried out in different seasons they are reported here, for the sake of convenience, in the above order.

EXPERIMENTAL

Experiment 1. *Effect of day-to-day variations in the botanical composition of the pasture*

For a period of approximately two months from mid-January 1953, samples were taken twice weekly from the bulked milk of the herd of The Dairy Research Institute (N.Z.). The herd comprised thirty-eight mixed-age, grade cows with a predominance of Jersey blood and at the time of the experiment the majority were in the seventh month of lactation. Throughout the experimental period they were rotationally grazed on typical mixed ryegrass-white clover-cocksfoot associations. Under the system of grazing practised the cows were offered a fresh break of approximately half an acre of pasture after each milking. Since each field on the farm was approximately an acre in area, the herd was shifted into a fresh field, and hence on to pasture of different botanical composition, on the average after every second milking.

In determining the botanical composition of the pasture point analysis was considered impracticable because of the rankness of the growth, and samples were, therefore, cut and sorted by hand, results being expressed on a dry-matter basis. From statistical analysis of the results of trials conducted to determine the variations between successive samples, a sampling technique was developed giving an accurate estimate of the percentage of clover in the feed consumed. Plots (24 plots 6×8 in. per field) were chosen at random. One half of each plot was cut approximately 1 in. above the ground before the field was grazed and the other half was cut similarly after grazing. From the weight of the sample and the weights of clover they contained, the percentage clover eaten was calculated.

Experiment 2. *Comparison of the effect on milk fat vitamin A potency of feeding clovers and ryegrass*

(*a*) *White clover versus ryegrass*

The comparison was made during March 1953, when, as shown by preliminary determinations, the vitamin A potency of the milk fat had reached its minimum value. Three sets of monozygotic twins (numbers 5, 6; 49, 50; 59, 80) from the herd of the Dairy Research Institute (N.Z.) were used. Following a 4-day pretreatment period when the

twins were grazing with the main herd a mixed pasture containing approximately 36% clover, the even-numbered animals of each pair were placed on a 'high-clover' pasture containing approximately 70% clover, and their twin mates were placed on a 'low-clover' pasture of ryegrass containing less than 18% clover. After 5 days the even-numbered animals were brought in from pasture, and for a further 7 days were stall-fed, *ad lib.*, clover cut from a pure stand. The odd-numbered animals remained on the same 'low-clover' pasture throughout. The pasture clover, like that used for stall feeding, was almost entirely white with a cyanide content of approximately 0·03% on a dry-matter basis (9) and was at a lush, rapidly growing stage. The pasture grasses were more mature, particularly those in the low-clover association.

Throughout the experiment individual milk samples were collected daily at the evening milking.

(*b*) *Red clover versus ryegrass*

Two pairs of monozygotic twins were used and the experiment was carried out during February 1955 when the vitamin A potency of the milk fat was again at a minimum. Following a 3-day pre-treatment period when the animals were run with the rest of the herd, two of the cows were fed, *ad lib.* for 8 days, red clover cut from a pure stand while their twin mates grazed a predominantly ryegrass pasture containing less than 20% clover on a dry-matter basis. Throughout the experiment, including the pre-treatment period and a 3-day post-treatment period, daily composite samples of night and morning milks were collected. Blood samples were also taken at approximately 2-day intervals. Both the clover and ryegrass were at a fairly advanced stage of growth when fed.

Experiment 3. *Effect of thiocyanate on the vitamin A potency of milk fat*

One outstanding difference between the white and red clovers used in the above experiments was their cyanogenic glucoside content, the former containing approximately 0·03% cyanide while the latter was cyanide free. When differences were found in the effect of the two clovers on the vitamin A potency of the milk fat the effect of dosing with thiocyanate, the probable detoxication product of cyanide in the ruminant, was investigated. This experiment was carried out in April 1955 when milk fat vitamin A potencies were increasing from the mid-summer minimum.

Two pairs of monozygotic twins were used and were grazed with the herd throughout the experiment. For the first 4 days two animals received in two equal doses, as a drench after the night and morning milkings, 2·7 g. potassium thiocyanate per day. The dosage rate was then increased for a further 6 days to 9·0 g. potassium thiocyanate per day, similarly administered. Their twin mates remained untreated throughout the experiment. During the experimental period, including a 3-day pre-treatment period, daily individual milk samples were taken at the evening milking and blood samples were taken at approximately 5-day intervals.

METHODS

Preparation of samples for analysis. After collection, the milk samples were stored at 0° C. for 12 hr. The cream was drawn off and churned and the butter obtained was melted and filtered at 60° C. and assayed shortly afterwards.

The possibility of a small error being introduced by this method of separation, due to incomplete recovery of the small fat globules, must be considered since Kon, Mawson & Thompson (10) have shown the proportion of carotene to be greater per unit volume in small fat globules than in large. Such error as has been introduced by the method, has, however, been shown in this laboratory to be small and may for practical purposes be disregarded.

Methods of analysis. The measurement of vitamin A alcohol and ester, carotene and xanthophyll in blood and butterfat was carried out using the extraction and separation procedures recommended by Thompson, Ganguly & Kon (15). Carotene, xanthophyll and vitamin A were estimated photoelectrically (the latter after reaction with antimony trichloride) in an EEL colorimeter based on the Evelyn design, readings being recorded by means of a mirror galvanometer and projection scale. The instrument was standardized for carotene and xanthophyll against a solution of pure carotene (British Chlorophyll Co.) in petrol ether, and for vitamin A against a standard vitamin A concentrate (Glaxo Laboratories (N.Z.) Ltd.).

Tocopherol contents of the fats were determined by the method of Quaife (11), iodine values by the standard method (12), and the unsaturated acid constituents using the methods and calculations described by Brice & Swain (13) and Brice, Swain, Schaeffer & Ault (14).

RESULTS

The percentage of clover in the feed eaten by the herd during the period of the first experiment ranged from 6 to 70%. The carotene content of the pasture varied from 200 to 380 μg./g. dry matter (mean 270 μg./g.) and the crude fibre from 20·5 to 29·5%. During the same period the fat from the bulked herd milk, sampled twice weekly, showed the following variations: vitamin A ester, 4·7–7·4 μg./g.; vitamin A alcohol, 0·12–0·14 μg./g.; carotene, 6·1–8·7 μg./g.; total vitamin A potency,* 26–36 i.u./g.; tocopherol, 44–59 μg./g.; oleic acid, 33·3–36·1%; iodine value, 35·1–39·6. The percentage of the vitamin A potency due to carotene varied from 33 to 49%.

These variations were larger than anticipated, but were obviously associated with the same factor or factors since highly significant correlations were established between total potency and iodine value (+0·605), and total potency and oleic acid percentage (+0·743). Significant correlations were also established between total potency and tocopherol content (+0·760) and between tocopherol content and carotene content (+0·756). The correlation between the vitamin A potency of the fat and the percentage of clover eaten over successive 12 hr. periods up to 5 days prior to milking was determined but without clear-cut results. Similarly no correlation could be established between either the amount of carotene or crude fibre in the pasture and the vitamin potency of the milk fat.

The effect of ryegrass versus white clover feeding (Exp. 2*a*) on the levels of vitamin A and carotene and on total vitamin A potency of the fat is shown in Fig. 1. The milk fat of the clover-fed twins showed an average decrease in vitamin A potency of 8 i.u./g. over the first 24 hr. while their twin mates on the low-clover pasture showed a corresponding increase of 4 i.u./g. These differences were due more to variations in the vitamin A content of the fat than to changes in the carotene content and were maintained throughout the experimental period. Variations in iodine value also occurred (Fig. 2) and these were

* Total vitamin A potency, i.u./g. fat = carotene μg./g. fat × 1·67 + vitamin A, μg./g. fat × 3·2(a).

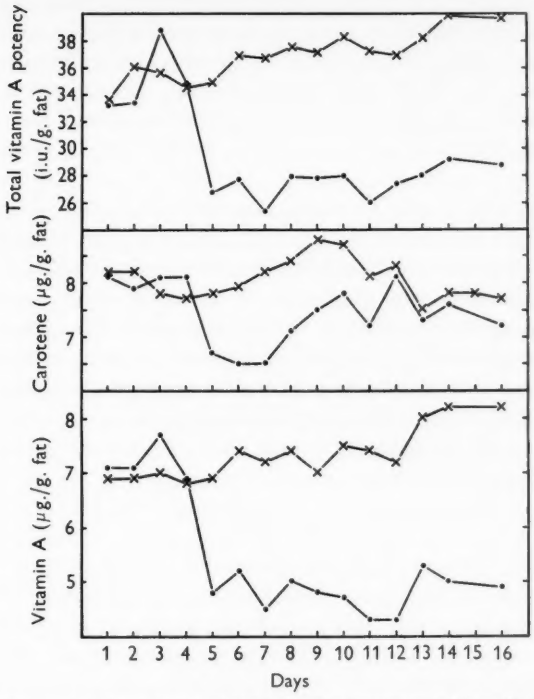

Fig. 1. Levels of vitamin A and carotene and total vitamin A potency in the milk fat of three pairs of monozygous twins fed clover (•—•) or ryegrass (×—×).

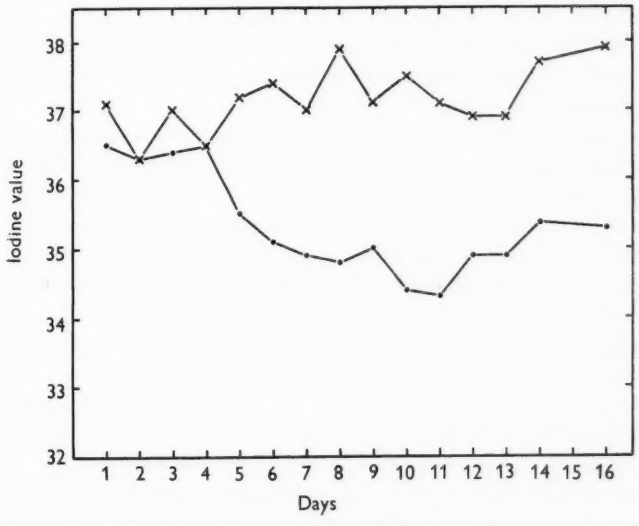

Fig. 2. Levels of iodine values in the milk fat of three pairs of monozygous twins fed clover (•—•) or ryegrass (×—×).

closely related to changes in total vitamin A potency. The average iodine value prior to treatment was 36·8, decreasing to a mean value of 35·4 in the clover-fed group and increasing to a mean value of 37·2 in the rye-fed group within 24 hr., and remaining thereafter relatively constant. Other milk fat components, investigated for only one set of twins, showed the following average levels for pretreatment, high-clover, and low-clover periods: tocopherol, 52, 44, 58 μg./g.; oleic acid, 34, 30, 35%; vitamin A alcohol, 0·15, 0·19, 0·17 μg./g.

In marked contrast the feeding of red clover versus ryegrass (Exp. 2*b*) had no effect on the levels of vitamin A in the milk fat, average levels of vitamin A alcohol ester, carotene and xanthophyll throughout the experiment being respectively 0·42, 5·7, 7·3 and 1·2 μg./g. fat. Similarly, blood levels were unaffected by treatment, average values for the same constituents being respectively 21, 2·4, 1050 and 55 μg./100 ml. plasma.

Likewise the dosing with thiocyanate (Exp. 3) did not affect the level of vitamin A or carotene in the milk fat or blood. Average values recorded throughout the experiment for vitamin A alcohol, ester, carotene and xanthophyll being 0·4, 6·4, 8·1 and 1·2 μg./g., respectively, in the milk fat and 29, 2·0, 1100 and 72 μg./100 ml. plasma in the blood.

DISCUSSION

The results from the first experiment confirm the low summer vitamin A potencies and iodine values reported previously for New Zealand butterfats(1, 2, 5). At the same time they also drew attention to the relatively large variations present in the vitamin A potency and iodine value of bi-weekly summer butterfat samples. None of these variations could be shown, however, to be due to changes in clover intake. In planning the experiments it was thought that the variations in clover content noted between fields would be sufficiently large to be reflected by significant changes in milk fat potency. Unfortunately it was not possible to establish a reciprocal relationship between the clover content of the pasture and the vitamin A potency of the fat produced from it. This was due most probably to the rapidity with which the level of clover intake varied, the effect of one level being completely masked by the effect of a widely differing level during the following period.

The results from Exp. 2*a* in which different pasture species were fed separately to monozygous twins clearly demonstrate, however, that botanical composition may have a marked effect on vitamin A potency and other milk fat constants, since diets high in clover significantly decreased fat potency, iodine number, and the contents of oleic acid and tocopherol, whereas diets low in clover had the opposite effect. That this effect was associated with white clover rather than red clover is strongly indicated from the results of Exp. 2*b* in which no difference could be found between the levels of vitamin A alcohol, ester, carotene, and xanthophyll in the blood plasma or milk fat of cows fed a diet high in red clover as opposed to a diet high in ryegrass.

At the present time the mechanism by which diets high in white clover depress carotene utilization from pasture and vitamin and provitamin secretion from the mammary gland is not clear. There would, however, appear to be four possibilities.

In the first place, McGillivray(5) has shown that the tocopherol content of clover is lower than that of grass sampled at the same time and that the level of tocopherol in pasture declines throughout the spring to reach a minimum in summer. These obser-

vations, together with the fact that he was able to raise the potency of summer fat by tocopherol supplementation, would suggest that a low level of tocopherol (or other anti-oxidant) in the pasture was perhaps at least partly responsible for the summer decline in potency.

In the second place, it was noteworthy that the white clover used in Exp. 2*a* was a high cyanide strain. It was thought highly probable, therefore, that cyanide, which is liberated from white clover by enzymic and microbial hydrolysis in the rumen, might be a further factor contributing to the problem. Following its absorption into the blood stream, it may possibly have some direct physiological effect or it may act indirectly after detoxication to thiocyanate. There are several *a priori* grounds on which to base the latter assumption. First, a very large proportion of the white clovers present in high-producing New Zealand dairy pastures are improved, high cyanide strains; moreover, it has been shown that the percentage of clover in typical pastures in this country follows a regular seasonal trend with maximum levels being present in the summer (8). Secondly, Coop & Blakely (16, 17) have shown the release, the absorption and the detoxication of cyanide to thiocyanate in the liver to be a comparatively efficient process in the sheep. Thirdly, Barker (18) and others (19, 20) have shown thiocyanate to possess anti-thyroid properties; and fourthly, Cama & Goodwin (21) and Chanda *et al.* (23, 22) have demonstrated a rapid decrease in the absorption of carotene in the rabbit, goat and cow following experimental induction of hypothyroidism with thiouracil. On these four grounds it is possible, therefore, that the decreased availability of carotene from summer pastures in New Zealand and the low levels of carotene and vitamin A in the butterfat at this time are associated with and are partly due to a lowered thyroid function resulting from higher than normal levels of blood thiocyanate. The results of preliminary experiments (Exp. 3), however, have failed to confirm this hypothesis. Other experiments are at present being planned, therefore, to further test this possibility.

In the third place, since carotene is a fat-soluble pigment it is reasonable to assume that its availability from pasture will depend to a certain extent on the amount and on the nature of the fat present in the pasture. Indeed, there is a considerable body of indirect evidence in the literature to support this hypothesis (24, 25, 26, 27). Krukovsky (27), for example, has presented evidence to show that the solubility of carotene in a fat is to a large degree dependent on its degree of unsaturation and from this has concluded that 'the efficiency of absorption of carotene by an animal from its feed might be influenced by the degree of unsaturation of the fat present in the feed'. Thus a low proportion of unsaturated acids in the feed would result in not only a low iodine value but also in a less efficient absorption of carotene from the feed and in a low carotene and vitamin A content of the milk fat. Unfortunately, the nature of the fat in New Zealand pastures has not been extensively investigated and there is as yet no confirmation of any seasonal change in its degree of unsaturation. Shorland (28), however, has shown New Zealand pastures to be characterized by a high content of C_{16} and C_{18} unsaturated acids of which by far the commonest is oleic. At the same time it has been demonstrated by Hansen & Shorland (29) and McDowell (30), working independently with New Zealand butterfats, that oleic acid, which is derived directly from the diet and is known not to be synthesized to any appreciable extent within the mammary gland (31, 32), varies in the fat throughout the season, reaching minimum levels in November and January. If then, as would appear likely, this trend reflects similar changes in either the level or availability of oleic acid in the pasture

and if, as has been suggested by Krukovsky (27), carotene is associated with oleic and other unsaturated acids during absorption, the low availability of carotene from summer pastures may be explained in part by a decrease in the content of oleic acid (or other unsaturated acids) in the pasture resulting perhaps from the drying up or hardening off of the pasture at this time or from changes in its botanical composition. Seasonal variations in the amounts and nature of the fats present in New Zealand pastures are at present being studied at the Plant Chemistry Laboratory of the Department of Scientific and Industrial Research, Palmerston North, and when the results of these investigations come to hand additional light will be shed on this particular aspect of the problem.

Finally, although in the present experiments it was shown that white clover may have a marked effect on the vitamin A potency of milk fat, the further possibility must be considered that the summer decline in potency may not be due to the effect of clover *per se* but may be caused by the accumulation of some specific substance (or group of substances) associated with the stage of development of the pasture as a whole at a time when clover happens to be the dominant species present. This is strongly suggested by the fact that, even though the odd-numbered twins during Exp. 2*a* were grazing pasture containing approximately the same amount of clover as average winter pastures (8), the vitamin A potency of their fat was considerably lower (and remained so throughout the experiment) than that of winter fat. This would seem to indicate that some factor in summer grass as well as in clover was interfering with the utilization of carotene. The finding that clover has a relatively greater effect on fat potency than grass might be due merely to the fact that it contains a somewhat greater concentration of the factor than grass.

Further experiments are at present being undertaken in this laboratory to investigate more fully the relationship between clover intake and vitamin A potency of butterfat and in particular the mechanism by which diets high in clover depress fat potency and will form the subject of subsequent communications.

SUMMARY

1. The effect of the level of clover intake on the vitamin A potency of milk fat has been studied, using Jersey cows from the herd of the Dairy Research Institute (N.Z.).

2. In preliminary experiments in which composite bi-weekly butterfat samples were obtained from the whole herd of thirty-eight cows no relationship could be established between level of clover intake and potency of fat produced. This was assumed to be due to the rapidity with which the level of clover intake varied, since under normal grazing management the cows were moved to a fresh paddock (which was often of widely different botanical composition from that previously grazed) after every second milking.

3. In later experiments in which three pairs of monozygous twins from the herd were used, a clear-cut relationship was established between clover intake and potency of fat produced and between clover intake and iodine value, oleic acid percentage and tocopherol content. Diets high in clover tended to depress total potency, iodine value, oleic acid percentage and tocopherol content of the fat, while diets low in clover had the reverse effect. The clover used in these experiments was mainly an improved strain of white clover containing approximately 0·03% cyanide. Repetitive experiments using improved strains of red clover produced negative results.

4. The mechanism by which diets high in clover depress fat potency, iodine value, oleic acid percentage and tocopherol content is not clear. It is suggested, however, that it may be due to (i) the low tocopherol content of clover relative to grass, (ii) the high HCN content of certain species, and (iii) the possibly lower oleic acid content. The possibility is also considered that it may be due to some factor (or group of factors) associated with the stage of development of the pasture as a whole at a time when clover happens to be the dominant pasture species.

5. Drenching cows with thiocyanate, the probable detoxication product of HCN in the ruminant, could not be shown to have any effect on the levels of vitamin A alcohol, ester, carotene, or xanthophyll in the blood and milk fat.

Some of the results of this investigation form a section of a thesis submitted by one of us (N.A.W.) in partial fulfilment of the requirements for the degree of M.Agr.Sc. of the University of New Zealand. The authors are indebted to the Department of Scientific and Industrial Research for a grant towards the investigation and to Mr M. R. Patchell and farm staff of The Dairy Research Institute (N.Z.) for assistance in collection of milk and blood samples. The technical assistance of Miss Fay Frecklington is gratefully acknowledged.

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657. FACTORS INFLUENCING THE VITAMIN CONTENT OF MILK FAT

III. SEASONAL CHANGES IN THE VITAMIN A AND CAROTENOID CONTENT OF THE BLOOD PLASMA AND MILK FAT OF COWS ON PASTURE

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(With 5 Figures)

Seasonal trends in the vitamin A and carotene content of New Zealand butterfat reported by Barnicoat (1) and McDowell & McDowall (2) have been discussed in the preceding papers of this series (3, 4). Under the conditions obtaining in most other dairying countries, the milk fat and blood plasma levels of these constituents show clearly defined seasonal fluctuations which can be related to the varying carotenoid intake of the cows. In a few cases (e.g. Lord (5), who also briefly reviews previous work) plasma and milk fat levels have been followed on the same cows at the same time, and it is apparent that the milk fat reflects fairly closely the changing levels in the plasma. While the seasonal trends in New Zealand milk fats cannot be ascribed to changes in the carotene intake (3), it seemed of interest to determine whether these somewhat unusual variations were also associated with similar changes in the level of vitamin A and carotenoids in the blood plasma. Further, it seemed that some importance might attach to the relative proportions of vitamin A alcohol and ester in the milk fat throughout the year since, according to Chanda & Owen (6), the level of vitamin A alcohol in the milk fat is an indication of the extent to which the animals are drawing on their hepatic vitamin A reserves. In the previous seasonal surveys (1, 2) the two forms of vitamin A were not differentiated.

EXPERIMENTAL

Six cows, predominantly Jersey, from the herd of The Dairy Research Institute (N.Z.) were used. The animals were fed out-of-doors on pasture as normal for the Institute herd. Milk samples were taken from night and morning milkings on three successive days to give a 3-day composite cream sample. This was churned in a laboratory churn (7) and a clear fat sample obtained by melting the butter and filtering in the dark at 60° C. These composite fat samples, together with blood plasma samples, were taken initially at weekly intervals and subsequently fortnightly. The collection of samples commenced late in August 1954, when most of the animals had been lactating for approximately one month, and continued until the end of lactation. During the same season commercial butter samples were obtained from a Manawatu factory at approximately fortnightly intervals and were assayed in the same way.

Vitamin A and carotenoids were determined in the milk fats and bloods essentially as described by Thompson, Ganguly & Kon (8); 2 g. of fat was found to be a convenient quantity for each assay. Tocopherol was estimated, using the method of Quaife (9).

RESULTS

One cow lactated for approximately 100 days only, and results from this animal are considered separately. Figs. 1 and 2 show the seasonal variations in the levels of vitamin A and carotenoids in the blood plasma and milk fat of the remaining five cows. Average values only are recorded, but all samples were assayed separately and all animals showed

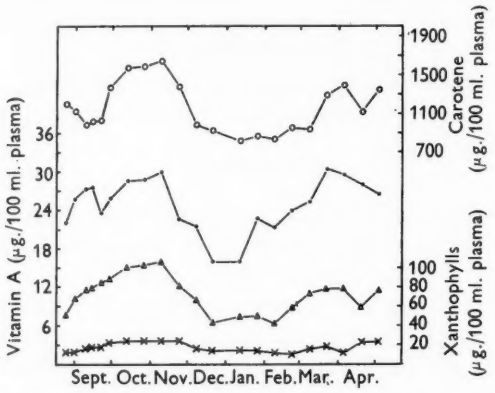

Fig. 1. Average levels of vitamin A and carotenoids in the blood plasma of five pasture-fed cows throughout one lactation. Vitamin A alcohol, •—•; vitamin A ester, × — × ; carotene, o—o; xanthophyll, ▵—▵.

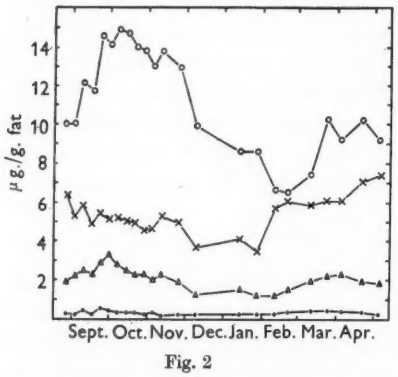

Fig. 2

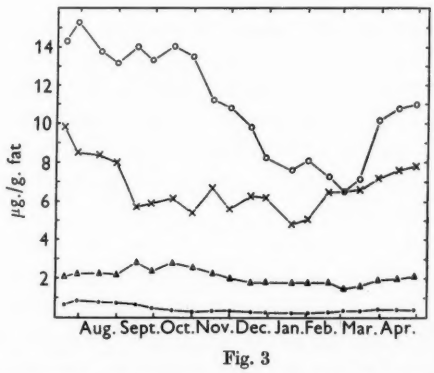

Fig. 3

Fig. 2. Average levels of vitamin A and carotenoids in the milk fat of five pasture-fed cows throughout one lactation. Vitamin A alcohol, •—•; vitamin A ester, × — × ; carotene, o—o; xanthophyll, ▵—▵.

Fig. 3. Levels of vitamin A and carotenoids in the fat from commercial butters supplied by a Manawatu factory. Vitamin A alcohol, •—•; vitamin A ester, × — × ; carotene, o—o; xanthophyll, ▵—▵.

similar trends. Corresponding values for the factory fats are given in Fig. 3. These factory fats show typical seasonal trends in carotene and total vitamin A. Vitamin A alcohol levels, although extremely low, showed a somewhat similar trend, decreasing from 0·8 μg./g. fat at the start of the season to 0·2 μg./g. in mid-summer. The significance of these lower alcohol levels is questionable. While the samples with the relatively higher vitamin A alcohol levels developed with the antimony trichloride reagent, a definite blue

colour typical of vitamin A, samples recorded as containing less than about 0·3 μg./g. developed in general no typical colour, even when assays were repeated using more than 2 g. fat or when alcohol fractions were combined. Further, little or no vitamin A could be detected in these samples when assayed spectro-photometrically using the Morton and Stubbs correction procedure (10). Above this level there was, in general, fair agreement between colorimetric and spectrophotometric figures, the latter averaging about 75% of the former. It would appear therefore that the colorimetric figures quoted for vitamin A alcohol are maximum values, that levels below about 0·3 μg./g. are probably fictitious, and that little significance can be attached to small variations in this range. Xanthophylls remained relatively constant, but showed a slight downward trend over the summer months.

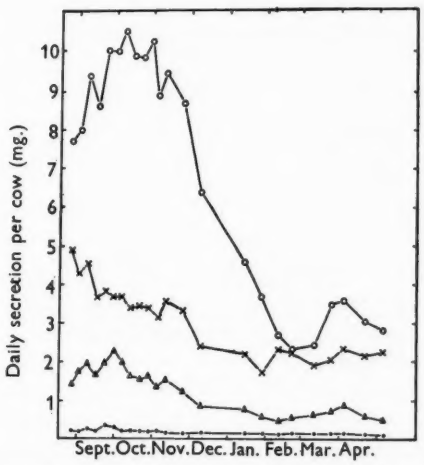

Fig. 4. Total average daily secretions of vitamin A and carotenoids for five pasture-fed cows. Vitamin A alcohol, •—•,; vitamin A ester, × — × ; carotene, o—o; xanthophyll, Δ—Δ.

In general, the levels of vitamin A and carotenoids in the milk fats of the individual cows showed seasonal variations similar to those in the factory butters. Carotene levels did, however, show an increase during September which was not apparent in the factory samples and the levels of vitamin A alcohol were lower in all cases, the maximum value recorded being 0·5 μg./g. in September. The trend in xanthophyll levels was also more clearly defined.

In the blood plasma of the five cows, marked seasonal variations were apparent in the levels of vitamin A alcohol, carotene and xanthophylls and to a lesser extent in vitamin A ester.

From the weekly production figures for the individual cows, the approximate daily secretions of vitamin A and carotenoids were calculated and the average values are recorded in Fig. 4. The fat yields from which these secretion figures are calculated are weekly averages based on daily milk yields and weekly determinations of fat percentage in a 2-day composite milk sample. Any day-to-day variation in fat yield could therefore introduce errors which, although relatively small, might account for much of the sample-to-sample fluctuation apparent in the results presented. The general picture is, however,

a high secretion of all constituents until November when decreasing concentration in the fat and decreasing fat yield caused a sharp drop to lower levels which were maintained fairly steadily to the end of lactation, further decreases in fat yield being offset by the rising concentrations in the fat.

Concentrations in the milk fat and approximate daily secretions of vitamin A and carotenoids for the sixth cow are shown in Fig. 5. Although lactation ceased in November when the levels in the milk fat of the other cows were decreasing, the levels in the fat of this cow showed an end-of-lactation increase similar to that occurring with the other cows in April and May. It is significant too that the extremely low fat yield of this cow throughout lactation was accompanied by high levels of vitamin A and carotene in the milk fat,

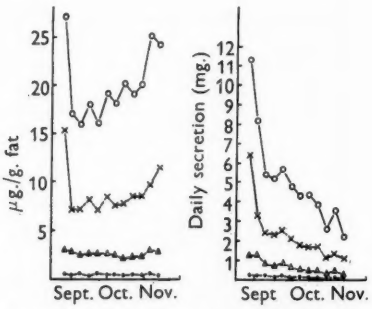

Fig. 5. Levels in the milk fat and total secretion of vitamin A and carotenoids for one pasture-fed cow which lactated for approximately 100 days only. Vitamin A alcohol, •—•; vitamin A ester, ×—×; carotene, o—o; xanthophyll, Δ—Δ.

the total secretion of these being only slightly less than for the other cows. Throughout lactation the blood plasma carotene level for this animal was considerably higher than average, reaching 3100 μg./100 ml. in early November, but up to the end of lactation plasma vitamin A and carotenoids showed the normal seasonal trends. After lactation ceased blood samples were collected at one or two monthly intervals only, but these also showed trends similar to the lactating cows, carotene decreasing from 3100 μg./100 ml. in November to 1000 μg./100 ml. in midsummer and vitamin A alcohol decreasing, not so markedly, from 30 to 24 μg./100 ml. over the same period.

Tocopherol was very closely correlated with carotene, both in the factory butters and the fats from individual cows. Average levels ranged from a maximum of 40 μg./g. fat in late spring to a minimum of 23 μg./g. in midsummer. Results are not reported in detail, as further work with an improved tocopherol assay method is being carried out in this laboratory to relate changes in tocopherol levels in the milk fat to changes in blood plasma.

DISCUSSION

From Fig. 1 it is apparent that marked seasonal variations occur in the levels of vitamin A and carotenoids in the blood plasma of cows on pasture under New Zealand conditions. Wide fluctuations in the levels of carotene and vitamin A in the blood plasma of cows have been reported by other workers (e.g. (5)), but these, like the attendant variations in the milk fat levels, are in the opposite direction to those reported here, and are associated

with differences in the carotene intake between periods of stall and pasture feeding, maximum levels occurring in summer when the animals are on pasture and minimum levels in winter during stall-feeding. Under New Zealand farming conditions, however, the carotene intake is high and relatively constant throughout the year (3), and the varying levels of vitamin A and carotene in the blood plasma, as in the milk fat, cannot be explained in terms of varying carotene intake. The marked decreases in the levels of carotene and xanthophylls, and the smaller but significant decrease in vitamin A ester, are consistent with a lowered availability or utilization of the carotenoids from the summer pastures. No explanation can be offered at this stage for the decrease in plasma vitamin A alcohol. This may be due in part, as discussed later, to an uptake by the mammary gland of plasma alcohol, but a similar, though not so marked, trend was apparent over the summer period in the plasma vitamin A alcohol of the non-lactating animal. These plasma vitamin A alcohol trends are being investigated further, particularly in relation to milk fat vitamin A.

In the light of our previous observations on the effect of clover on the vitamin A potency of milk fat (4), it is significant to note that during November when the marked decreases in plasma and milk fat vitamin A and carotene levels occurred, the pasture, owing to the particular climatic conditions obtaining at that time, showed an equally marked and rapid change from ryegrass to white clover dominancy.

The correlations between plasma and milk fat carotenoid levels reported by other workers (e.g. (5,11)) were also apparent in the present investigation, milk fat levels reflecting changing plasma carotenoid levels very closely throughout the season.

In the case of vitamin A, comparable seasonal trends also occur in the milk fat and plasma levels, but here the picture is complicated by the fact that, although the free and esterified forms of vitamin A are present in both milk fat and blood plasma, in the former the esterified form predominates, while in the latter the reverse is normally true. Plasma carotene must be the source of milk fat carotene, but the vitamin A in the milk fat could be derived partly or completely from either the plasma vitamin A alcohol or ester. Experiments by Chanda and co-workers (6,12) suggest that milk fat vitamin A arises from both the alcohol and ester forms of vitamin A in the plasma, the former being replaced from hepatic stores and the latter being taken up directly by the mammary gland without passing through the liver. The relative importance of these two sources of vitamin A would appear to depend on the level of vitamin A ester in the plasma. If this is low or entirely absent from the plasma, there is an increased utilization of hepatic vitamin A through the plasma vitamin A alcohol. Incomplete esterification in the gland of this increased amount of alcohol form would then explain the higher levels of vitamin A alcohol found by these workers in the milk fat of cows on a carotene-free diet.

Applying this theory to the seasonal trends observed in New Zealand milk fats, the slight decrease in plasma ester levels over the summer months might be sufficient to explain the decreased milk fat vitamin A levels. A further suggestion that the animals are striving to maintain milk fat vitamin A levels at the expense of hepatic reserves, and that the release of this hepatic vitamin A is not as rapid as its removal from the blood by the mammary gland, is not incompatible with the plasma vitamin A alcohol picture, but on the basis of the work of Chanda *et al.* (6,12), some attendant increase in milk fat vitamin A alcohol might be expected as the plasma ester level decreased and the dependence on plasma alcohol increased. No such increase in vitamin A alcohol was

apparent, however, in either the fats from the individual cows or in the factory samples, the levels showing, if anything, a decrease over the summer months.

McDowell (13) has recently drawn attention to a number of differences between the results for herds, where calving would be restricted to a relatively short period, and factory samples, where the spread of calving dates would be much wider and where seasonal trends during winter and spring months would be considerably modified by the inclusion of colostral and early post-colostral fat and end-of-lactation fat from animals which calved late in the previous season. These differences are, however, most apparent prior to the period covered in this present investigation, and the results presented here confirm McDowell's findings that from October until the end of the season there is little difference between factory and individual animal results.

The comparable levels of carotene in the milk fat of spring and autumn calving herds during the winter and early spring, also reported by McDowell (13), provides further and conclusive evidence that the increase at this time is a seasonal rather than lactational effect. It is significant, however, that there is less evidence regarding vitamin A levels in milk fat at this time, and the differences between herd and factory samples suggest that the higher levels in the latter may be due largely to lactational effects. The upward trends in vitamin A and carotene levels in the autumn (April and May), as most cows in typical New Zealand herds are nearing the end of their lactation, could be regarded, at least in the case of carotene, as the start of the seasonal increase which results in the high levels during July to November. The results presented here, however, would suggest that these autumn increases in vitamin A and carotenoids may be due more to lactational than seasonal effects. Total secretion of these constituents remains relatively constant over this period, the decreasing fat yield resulting in, or at least being associated with, an increased concentration in the fat. The similar end-of-lactation increase shown by the animal which ceased lactation in November (Fig. 5) was clearly a lactational and not seasonal effect, and again total secretion remained relatively constant.

SUMMARY

1. The levels of vitamin A alcohol and ester, carotene and xanthophyll in the blood plasma of six cows, grazed out-of-doors on pasture under typical New Zealand conditions, have been followed throughout one lactation. The same constituents have also been followed in the milk fats of these animals and in commercial factory butters over the same season.

2. The seasonal trend in the vitamin A and carotenoid content of New Zealand milk fat is a reflexion of the changing level of these substances in the blood plasma.

3. The decreased level of carotene, xanthophyll and vitamin A ester in the blood plasma over the summer months is consistent with a decreased absorption or utilization of carotenoids over this period.

4. Throughout the year the vitamin A in the milk fat is predominantly in the ester form with no increase in vitamin A alcohol indicative of any increased utilization of hepatic vitamin A reserves.

The author is indebted to the Department of Scientific and Industrial Research for a grant towards this investigation; to Mr M. R. Patchell and the farm staff of The Dairy

Research Institute (N.Z.) for assistance in the collection of milk and blood samples; to Dr F. H. McDowall, Mr A. K. R. McDowell and the staff of the Chemistry Laboratory, The Dairy Research Institute (N.Z.), for the preparation of the fat samples and for the supply of factory butters. The assistance of Miss Fay Frecklington and Miss Jane Monroe with part of the experimental work is also acknowledged.

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658. FACTORS INFLUENCING THE VITAMIN CONTENT OF MILK FAT

IV. THE SOURCE OF MILK FAT VITAMIN A AND CAROTENE

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(With 2 Figures)

During the course of investigations into factors affecting the vitamin content of New Zealand milk fats (1, 2, 3) the need for further information about the source of the milk fat vitamin A and carotene became apparent. Experiments were therefore carried out to investigate the relationship between the levels of carotene and vitamin A ester in the blood plasma and milk fat, and to assess the relative contributions of vitamin A of hepatic origin (through the blood plasma vitamin A alcohol) and vitamin A of immediate dietary origin (through the blood plasma vitamin A ester) to the vitamin A content of the milk fat. It is well recognized that for the production of milk fat of high vitamin A potency, cows are more dependent on a daily carotene or vitamin A intake than on liver reserves of vitamin A (e.g. (4)). For other species, relationships between dietary and milk fat vitamin A have been studied (e.g. (5)) and aspects of the problem have recently been investigated in cows by Chanda and co-workers (6, 7, 8). It seemed, however, that further information obtained under New Zealand conditions would enable a more precise interpretation of the seasonal variations already reported (3, 9, 10, 11) in the levels of vitamin A and carotenoids in the milk fat and blood plasma of pasture-fed cows in this country.

EXPERIMENTAL

The cows used were predominantly Jersey from the herds of The Dairy Research Institute (N.Z.) and Massey Agricultural College. Unless otherwise stated, all cows were milked normally twice daily, milk samples being collected at the evening milking.

In the first experiment, carried out in December, two cows, which had been fed normally on pasture, were brought indoors and fed, for 11 days, a carotene-free diet consisting of linseed meal, crushed oats and bran in equal quantities and straw *ad lib.* as roughage. At the end of this period they were returned to pasture. Milk and blood samples were taken throughout the experiment.

In the second experiment, carried out in August, the effect on the milk fat levels of a single dose of vitamin A was investigated. The cows were on pasture throughout the experiment and were dosed orally with varying levels of vitamin A.* Milk samples were collected at intervals and, in the case of one cow, simultaneous milk and blood samples were taken at frequent intervals following the dose of vitamin A.

* The vitamin A was administered as an emulsion supplied by Nicholas Products Ltd., Wellington, and containing approximately 8 mg. vitamin A per gram.

Milk fats were prepared for assay essentially as described in Part III of this series (3) and vitamin A and carotenoids were estimated in the milk fats and blood plasmas using the method of Thompson, Ganguly & Kon (12).

RESULTS

(1) *Effect of carotene-free diet*

Average vitamin A and carotenoid levels in the milk fat of the two cows transferred from pasture to the carotene-free diet are given in Fig. 1. A problem inherent in this type of experiment in this country is the relatively short time during which normal cows can be maintained on pasture-free diets. Under New Zealand conditions of pasture feeding out-of-doors throughout the year, the cows have not previously experienced stall-feeding nor

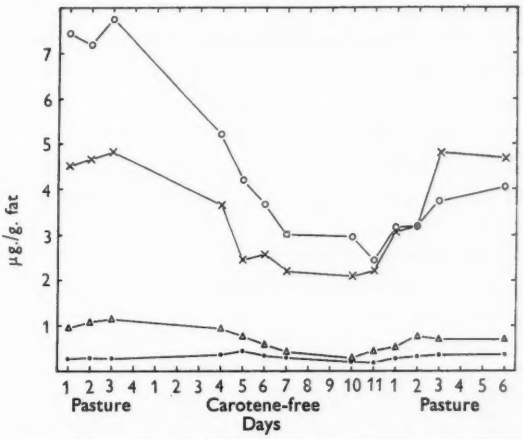

Fig. 1. Vitamin A and carotenoids in the milk fat of two cows transferred from pasture to a carotene-free diet and subsequently returned to pasture. Vitamin A alcohol, ●—●; vitamin A ester, × — × ; carotene, o—o; xanthophyll, Δ—Δ.

are they used to the types of feed necessary in carotene-free diets. In a number of cases they refused to eat the feed at all or tired of it after a few days, and a frequent reaction to the unusual conditions was a decrease in production which could mask the effect of dietary treatment. Throughout the experiment reported here, however, the animals ate normally and production was maintained, but the stall-feeding period had to be terminated after the 11 days as the animals showed signs of tiring of the diet.

Both vitamin A ester and carotenoids decreased sharply over the first 5–7 days on the carotene-free diet and were then maintained at a steady level until the cows were returned to pasture. Vitamin A alcohol remained at a low level throughout the experiment and, as previously pointed out (3), little significance can be attached to the small increase from 0·25 μg./g. fat to 0·45 μg./g. which occurred over the first 5 days on the carotene-free diet. Over the experimental period vitamin A ester and alcohol remained relatively constant in the milk fat of a group of pasture-fed control cows, but there was some decrease in carotenoid levels. At the start of the experiment the milk fat of these cows contained 10·8 μg. carotene per gram, and this decreased uniformly to 7·6 μg./g. Allowing for this decrease, the levels of carotene in the milk fat of the treated cows had returned almost to

normal during the 6-day pasture-feeding period at the end of the experiment and had reached a steady level of 5·6 μg./g. when the next samples were taken after an interval of one week.

The changes in the levels of vitamin A ester in the milk fat of the two treated cows reflected very closely the changes which occurred in the blood plasma vitamin A ester. These plasma levels decreased from an average of 2·9 μg./100 ml. during pasture feeding to levels too low to be detected after 7 days on the carotene-free diet and returned to normal after 3 days on pasture. Plasma levels of carotene and vitamin A alcohol showed only small sample-to-sample fluctuations with no treatment effects throughout the experiment. Average levels for plasma vitamin A alcohol and carotene were 24 and 1520 μg./100 ml. respectively. Xanthophyll levels decreased from an average of 98 to 69 μg./100 ml. plasma during the carotene-free period.

Similar results to those reported in Fig. 1 were obtained when the experiment was repeated later with two cows whose hepatic vitamin A stores had been increased by dosing with 500 mg. vitamin A per day for 14 days some 2 weeks prior to the start of the carotene-free period.

(2) *Effect of a single dose of vitamin A*

Levels of vitamin A and carotenoids in the milk fat of cows following a single oral dose of vitamin A and of control cows are shown in Table 1. In all cases there was a marked

Table 1. *The effect on vitamin A and carotenoids in the milk fat of a single oral dose of vitamin A*

			Milk fat			
			Vitamin A			
No. cows	Dose mg.	Time after dose (days)	Alcohol (μg./g.)	Ester (μg./g.)	Carotene (μg./g.)	Xanthophyll (μg./g.)
4	—	0*	0·49	5·5	13·9	4·2
		1	0·50	5·5	14·5	4·4
		3	0·47	5·4	14·2	4·4
		5	0·50	5·6	14·8	4·5
		7	0·47	5·7	14·2	4·5
		14	0·48	5·5	14·7	4·6
		21	0·43	5·1	13·8	4·4
2	750	0*	0·33	5·8	12·0	1·5
		1	0·66	23	11·3	1·4
		3	0·30	8·1	10·3	1·4
		5	0·33	7·9	10·5	1·4
		7	0·27	7·0	10·4	1·3
		14	0·42	7·4	9·6	1·4
		21	0·36	5·2	12·4	1·4
2	1500	0*	0·47	5·2	17·8	2·7
		1	1·31	43	15·8	2·7
		3	0·55	13	15·6	2·3
		5	0·42	8·3	14·9	2·4
		7	0·38	7·0	14·4	2·3
		14	0·48	7·0	12·9	2·3
		21	0·54	4·9	15·3	2·4
4	3000	0*	0·48	5·5	14·7	4·6
		1	3·30	132	11·8	3·5
		3	0·72	15	10·3	3·0
		5	0·65	9·9	13·2	3·3
		7	0·54	8·4	13·5	3·6
		14	0·58	6·1	13·6	4·1
		21	0·44	5·3	14·0	4·2

* These figures are the mean of three predosing samples.

increase in vitamin A ester and a smaller increase in vitamin A alcohol 24 hr. after the dose. The levels decreased rapidly over the next few days, but in the case of the ester form did not return completely to normal for 14–21 days. This slow return to normal is surprising, and although the bulk of the dose reached the intestine fairly rapidly it would appear that small amounts of vitamin A were retained in the rumen and only released slowly over a considerable period of time.

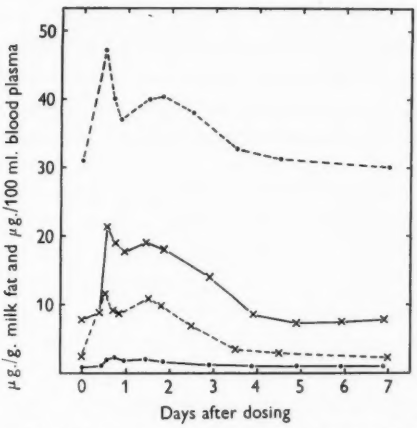

Fig. 2. The relationship between blood plasma and milk fat vitamin A ester and alcohol levels following a single dose of vitamin A (750 mg.). Plasma vitamin A alcohol, •- - -•; plasma vitamin A ester, ×- - -×; milk fat vitamin A alcohol, •—•; milk fat vitamin A ester, ×—×.

There was an associated decrease in carotene and to a lesser extent xanthophyll levels, the effect still being apparent after 14 days. The levels of vitamin A and carotenoids in the milk fat of the control cows remained relatively constant throughout the experiment.

The relationships between blood plasma and milk fat vitamin A alcohol and ester levels following a single dose of 750 mg. vitamin A are shown in Fig. 2. The cow was milked out as completely as possible at each milking.

DISCUSSION

From the experiments reported here it is clear that a close relationship exists between the levels of vitamin A ester in the milk fat and blood plasma. While on the carotene-free diet the level of vitamin A ester reflected the decreasing level of ester in the blood plasma, and despite the relatively short period over which it was possible to feed this diet, appeared to have reached a steady level of about 2·2 μg./g. fat. At this time vitamin A ester was no longer detectable in the blood plasma and the milk fat ester was presumably derived from plasma vitamin A alcohol. That there was no associated decrease in plasma vitamin A alcohol lends some support to our previous suggestion that the decreased level of vitamin A alcohol in the blood plasma of pasture-fed cows during midsummer in New Zealand is a seasonal trend not connected with lactation (3). During the carotene-free period, in contrast to the findings of Chanda *et al.* (6, 7, 8), there was no significant increase in the milk fat vitamin A alcohol.

The cows whose stores of vitamin A had been increased by prior dosing with vitamin A

also secreted during the period on a carotene-free diet, milk fat with a vitamin A ester level of only about 2·2 μg./g., so that this level is apparently independent of the vitamin A stores of the animals. It must be derived from the plasma vitamin A alcohol, and it is perhaps significant that an examination of the vitamin A ester levels reported in the seasonal survey (3) shows, throughout the season, an excess of milk fat vitamin A ester (in μg./g. fat) over plasma vitamin A ester (in μg./100 ml. plasma) of 2·7 with a standard deviation of ± 1·4, a relationship which is not inconsistent with the view that, irrespective of the plasma vitamin A ester levels, some milk fat vitamin A ester (1·6–3·8 μg./g. fat) has its origin in vitamin A of other than immediate dietary origin.

Over long periods clear relationships have been established between the levels of carotene in the blood plasma and the milk fat (e.g. (3,13)). Over the short period reported here, however, the marked drop in milk fat carotene (Fig. 1) was not associated with any significant decrease in blood plasma carotene. Thus, while carotene in the blood plasma remained at about 1520 μg./100 ml., the level of carotene in the milk fat decreased from 7·5 to 2·8 μg./g. fat. It seems reasonable therefore to postulate the existence of two forms of carotene in the blood plasma, one a normal circulating form making up the bulk of the plasma carotene, corresponding to the plasma vitamin A alcohol, and, like it, probably intimately associated with the plasma proteins, and the other a transport form, corresponding to the plasma vitamin A ester, and present when the animals are on a carotene-containing diet and at levels which reflect the immediate carotene intake of the animals. This transport form of carotene would be associated with vitamin A ester and dietary fat in the chylomicrons, and the direct uptake of these by the mammary gland (14) would account for the bulk of the carotene and vitamin A in the milk fat. The other form of carotene, like plasma vitamin A alcohol, apparently contributes little to milk fat levels, and in the experiments reported here would account for the level of 2·8 μg./g. fat at the end of the carotene-free period. Subsequent experiments have, however, shown that over longer periods of carotene deprivation, this form also decreases, due to utilization or destruction, and hence the relationships, over a longer term, between carotene intake, total plasma carotene and milk fat carotene.

It is now clearly established that in cattle, as in other species, vitamin A is transported from the intestines to the storage organs mainly in the esterified form (e.g. (15,16)). A single dose of vitamin A would therefore cause a marked transitory rise in blood plasma vitamin A ester, and the results presented in Table 1 show that a similar rise occurred in the level of vitamin A ester in the milk fat reflecting qualitatively but not quantitatively the magnitude of the dose. Unfortunately these milk fat vitamin A ester increases cannot be related directly to plasma ester levels, since from Fig. 2 it is apparent that during the absorption of the vitamin there is some increase in the level of vitamin A alcohol as well as ester in the blood plasma. In the case of this animal, however, milk fat vitamin A ester was more closely correlated with plasma ester than with plasma alcohol, the average relationship being: milk fat vitamin A ester in μg./g. = 1·48 plasma vitamin A ester in μg./100 ml. + 4·2, which further suggests that the milk fat vitamin A ester is not all derived from plasma vitamin A ester, even when the levels of the latter are high.

Deuel, Hallman, Johnston & Mattson (17) have shown that repeated dosing with vitamin A results in a lowering of the carotene content of the milk fat. A slight decrease in carotene levels is also apparent following the single dose of vitamin A (Table 1) and is due presumably to selective absorption of the vitamin A.

SUMMARY

1. The effect of varying levels of vitamin A ester in the blood plasma of cows on the levels of vitamin A ester in their milk fat has been investigated.

2. The milk fat levels reflect plasma levels, but it appears that, irrespective of the immediate carotene or vitamin A intake of the animals or of their liver reserves, some of the milk fat vitamin A ester is derived from vitamin A of other than immediate dietary origin.

3. It is suggested that the bulk of the plasma carotene contributes little to the level of carotene in the milk fat. The latter derives mainly from the carotene of immediate dietary origin which, during transport from the intestines to the storage organs, is associated with dietary fat and vitamin A ester in the chylomicrons.

The author is grateful to the Department of Scientific and Industrial Research for a grant towards this investigation; also to Mr M. R. Patchell and the farm staffs of The Dairy Research Institute (N.Z.) and Massey Agricultural College for assistance with the feeding and dosing of the animals and the collection of milk samples. The assistance of Miss Fay Frecklington with some of the experimental work and of Mr A. C. Glenday with statistical examination of the results is also acknowledged.

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659. FACTORS INFLUENCING THE VITAMIN CONTENT OF MILK FAT

V. CHANGES IN VITAMIN A AND CAROTENOID CONTENT OF THE BLOOD PLASMA OF COWS AND HEIFERS NEAR PARTURITION IN RELATION TO SECRETION OF THESE SUBSTANCES IN THE MILK FAT

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(With 4 Figures)

A decrease in the level of carotene in the blood plasma of cows around parturition was reported by Gallup & Kuhlman (1) in 1941. Subsequent workers, both in Great Britain (2,3) and in the United States (4,5,6,7), have confirmed and extended this observation to show that plasma levels of both carotene and vitamin A decrease to a variable extent at or immediately following parturition and return slowly to normal over a period of up to 3 weeks. Walker, Thompson, Bartlett & Kon (2) showed that the magnitude of the decrease depended to some extent on the vitamin A intake prior to parturition, being less marked in those animals supplemented with vitamin A. It seemed of interest, therefore, to investigate the blood picture at parturition under New Zealand conditions of pasture feeding and hence high carotene intake and to attempt to relate any decrease, similar to that reported overseas, to the secretion of carotene and vitamin A in the colostrum and early milk.

EXPERIMENTAL

Two groups of animals, predominantly Jersey, from the herd of The Dairy Research Institute (N.Z.) were used. The animals in each group were selected for the closeness of their expected calving dates and were all grazed on similar pasture, predominantly rye-grass, before and after calving. There was no apparent change in composition or nature of the feed available throughout the period covered, and the carotene intake from the pasture was of the order of 4–7 g. per day. One group comprised four cows and the other six heifers. All were spring-calvers, the cows calving between 13 and 24 June, and the heifers between 22 July and 9 August.

Blood samples were taken every second day, from 2 to 3 weeks before the expected calving date and for a corresponding period after calving. Total milk and fat yields were recorded and samples taken for assay daily for the first week of lactation and subsequently at 2-day intervals until the milk fat vitamin A and carotene levels had fallen to normal. Vitamin A ester, alcohol and carotene in milk fats and bloods were assayed as described in Part III of this series (8), using the chromatographic separation on alumina of Thompson, Ganguly & Kon (9). During the first 4 days of lactation fat was extracted from the colostrum samples, using the method of Olson, Hegsted & Peterson (10).

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Serum samples, collected at the same time as blood plasmas, were separated by paper electrophoresis into component proteins, using the methods of electrophoresis and staining sensibly as described by Block, Durrum & Zweig (11).

RESULTS

Blood levels

Three of the four cows showed consistently similar trends in plasma vitamin A alcohol and ester, carotene and xanthophyll levels. Average results for these animals are shown in Fig. 1. After a sharp rise commencing 3–4 days prepartum, carotene fell rapidly, reaching a minimum value 3 days post-partum and then returning slowly to normal over the next 5–10 days. Vitamin A ester, and to a lesser extent xanthophyll, showed a

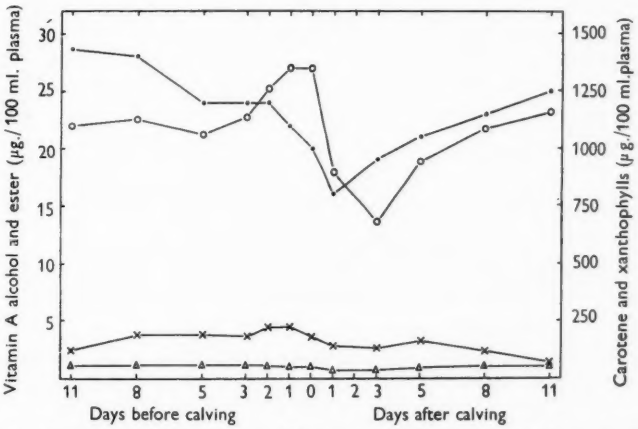

Fig. 1. Vitamin A and carotenoids in the plasma of three cows 11 days before and after parturition. Vitamin A alcohol, •—•; vitamin A ester, × — × ; carotene, o—o; xanthophyll, ∆—∆.

somewhat similar trend. Vitamin A alcohol did not show any prepartum rise and, after a small decrease some 7 days before calving, fell more rapidly over the 2 days preceding parturition, to reach a minimum 1 day after calving. It returned to normal at about the same rate as carotene. Plasma vitamin A and carotenoid levels prior, or subsequent, to the 22-day period covered in Fig. 1 were remarkably constant.

In contrast to those in the cows, the levels of vitamin and carotenoids in the plasma of the heifers fluctuated somewhat irregularly over the whole period. The average changes near parturition are presented in Fig. 2 and it is apparent that, although of a somewhat similar nature, they are much less marked than in the case of the cows.

Milk fat levels

Average levels of vitamin A and carotenoids in the colostrum and early milk of the three cows and six heifers are shown in Fig. 3. The concentration of all components was higher in the colostrum of heifers than in that of cows. The concentrations in the milk fat also remained high for longer in the heifers, reaching steady levels after about 10–14 days as compared with 6–7 days for the cows.

Total secretion

During the early stages of lactation the two groups differed markedly in total daily secretion, particularly of vitamin A ester and carotene. This was due to the higher milk fat production of the cows during the colostral period, and after the heifers were fully in

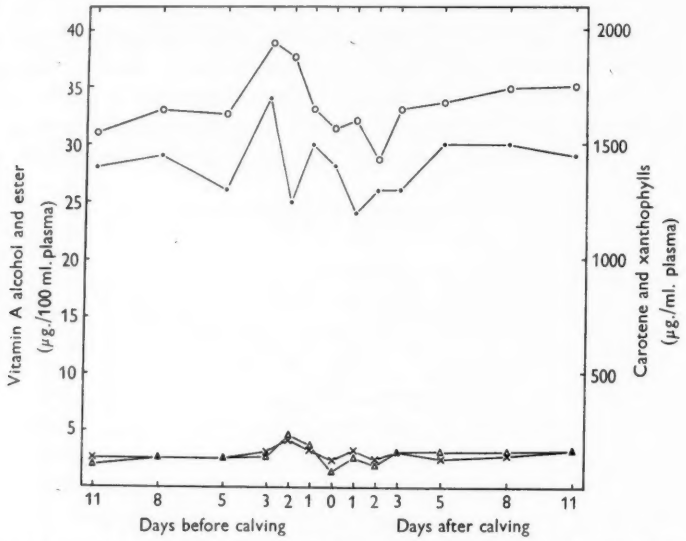

Fig. 2. Vitamin A and carotenoids in the plasma of six heifers 11 days before and after parturition. Vitamin A alcohol, •—•; vitamin A ester, ×—×; carotene, o—o; xanthophyll, ▵—▵.

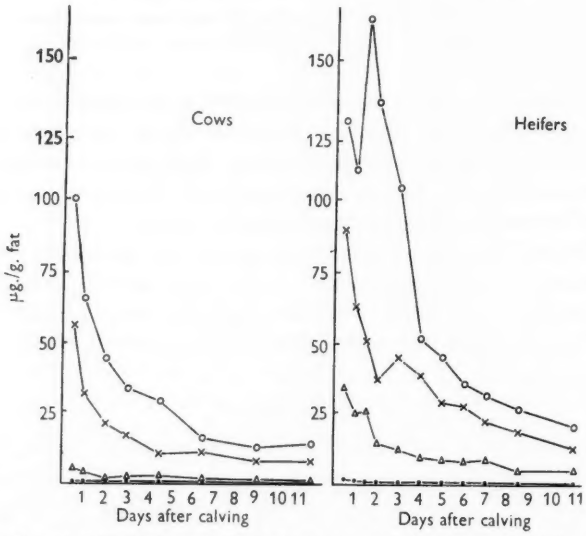

Fig. 3. Vitamin A and carotenoids in the milk fat of three cows and six heifers, for 11 days after parturition. Vitamin A alcohol, •—•; vitamin A ester, ×—×; carotene, o—o; xanthophyll, ▵—▵.

milk total secretion became comparable in the two groups. With the cows, maximum daily secretion occurred on the first day, whereas with the heifers this did not occur until the third day after calving. Average results for the two groups are presented in Fig. 4. In this, as in all other graphs, the same scale has been used for the cows and heifers to enable direct comparison.

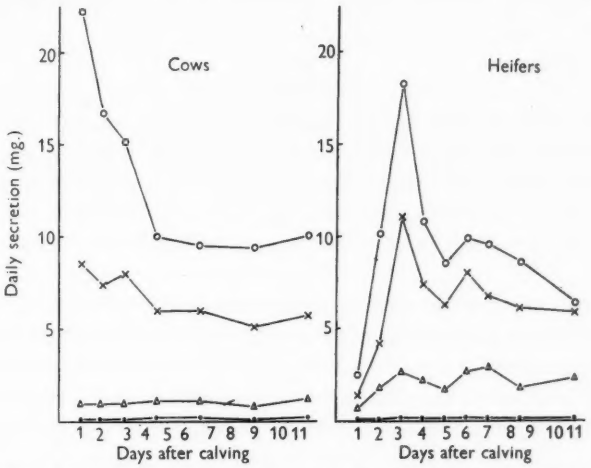

Fig. 4. Daily secretion of vitamin A and carotenoids in the milk of three cows and six heifers, for 11 days after parturition. Vitamin A alcohol, •—•; vitamin A ester, ×—×; carotene, o—o; xanthophyll, Δ—Δ.

The fourth animal in the group of cows was abnormal and results have not been included in any of the averages. This cow did not produce milk after calving and did not come into production at all until secretion was stimulated 4 days later by injections of Oestradiol monobenzoate. The secretion then produced resembled colostrum, and although the fat was somewhat lower than normal in vitamin A and carotene these levels were maintained over a longer period as shown in Table 1. Maximum secretion of vitamin A

Table 1

Days after calving	Vitamin A ester (μg./g. fat)	Carotene (μg./g. fat)
4	39	43
5	49	49
6	28	52
7	21	42
8	19	40
9	16	31
11	14	25
13	10	17
17	8	12

and carotene did not occur immediately, as in the case of the other cows, and was attained only 2–4 days later. The blood plasma of this animal showed no decrease in vitamin A or carotene levels at parturition, but showed the typical decrease 10 days after calving, i.e. at about the time when the secretion of vitamin A and carotenoids in the milk was at a maximum.

In the study of serum protein changes near parturition, after electrophoresis the

stained paper strips were compared visually and in some cases scanned photoelectrically. Throughout the period covered, no marked changes either in total protein concentration or in distribution of individual proteins could be detected. The investigation was of a somewhat exploratory nature, however, and more refined techniques would have been necessary to determine whether or not minor changes in pattern, which could have been related to the changes in vitamin A and carotenoid blood levels, did in fact occur.

DISCUSSION

From the results presented here it is apparent that the high carotene intake of cows under New Zealand farming conditions does not prevent the marked drop in plasma vitamin A and carotene near parturition. Although the carotene intake in our experiment was three to five times as great, this is in agreement with the work of Walker *et al.* (2) who showed that a supplement of 1·5 mg. carotene per day prepartum did not prevent the drop in plasma levels.

This temporary decrease in carotene and vitamin A could result from hormonal changes associated with parturition or from the abnormally large drain, due to colostrum secretion, on plasma vitamin A and carotene. The different rate of vitamin A and carotene secretion by the cows and heifers during the first few days of lactation and the behaviour of the cow which did not lactate immediately following parturition support the latter suggestion.

In the two groups of animals the decrease in carotene and vitamin A plasma levels is most apparent over the first 3 days post-partum. For the cows, the average total secretion of carotene during this period was 54 mg., while the corresponding figure for the heifers was only 31 mg. The drop in plasma carotene from prepartum maximum to post-partum minimum was 50% in the cows and, at the most, 25% in the heifers. Similar considerations apply to vitamin A in the milk fat and vitamin A alcohol in the plasma. Taking the same 3-day period, the total average secretions were 24 and 16 mg. and the corresponding decreases in plasma vitamin A alcohol were approximately 40 and 25% for the cows and heifers respectively.

For vitamin A secretion, however, the picture is complicated by the presence of both vitamin A alcohol and ester in the blood, and the above arguments presuppose esterification of vitamin A alcohol in the mammary gland. While plasma vitamin A ester may be the main source of milk fat ester (12), there seems ample evidence of the ability of the mammary gland to esterify plasma alcohol fairly completely (13). If esterification also occurs in the synthesis of colostrum, the process must be remarkably efficient, since very little vitamin A alcohol appears in colostral fat, the levels found in this investigation ranging from a maximum of 1·6 μg./g. of fat on the first day of lactation to a steady level of 0·3–0·5 μg./g. of fat about the 8th–10th day. Thus vitamin A alcohol contributed less than 2% to the total vitamin A present at the start of lactation and about 5% or less at later stages. The level of vitamin A alcohol in the colostrum of the cows was about one-third that in the heifers. It is of interest, however, to note that during the course of this investigation, two prepartum samples were obtained. In one of these, obtained 1 day before calving, 30% of the vitamin A was present in the alcohol form, and in the other, obtained 4 days before calving, 10% was present as the alcohol. These abnormally high alcohol levels in prepartum milk samples are being further investigated.

With the one animal which failed to lactate normally, the effects of parturition are separated by 4 days from the effects of lactation. Allowing for the fact that maximum secretion of vitamin A and carotene did not occur immediately lactation was established, the decrease in plasma levels coincides reasonably closely with the maximum secretion of carotene and vitamin A in the colostrum.

On the basis of this evidence, particularly for carotene, it seems reasonable to infer that, contrary to the suggestion of Goodwin & Wilson (3), the drop in plasma levels is due to the abnormally large uptake of carotene and vitamin A by the mammary gland during the colostral period, rather than to any physiological disturbances directly associated with parturition. Other blood components in which decreases have been reported near parturition such as lipids (14), tocopherol (15) and calcium and phosphorus (14) are also all secreted in large quantities in the colostrum and the same explanation may apply in these cases also.

SUMMARY

1. Plasma levels of vitamin A and carotenoids prior to and following parturition have been followed in groups of cows and heifers maintained under typical New Zealand farming conditions.

2. Despite the high carotene intake prior to calving, the cows, and to a lesser extent the heifers, showed decreases in plasma vitamin A and carotenoid level at or near to parturition, the decreases in the levels for the cows being of the same order as those reported in Great Britain and the United States.

3. Although the concentration of vitamin A and carotenoids was higher in the colostrum of heifers than of cows, the total daily secretion of these components by the cows was much greater over the first few days of lactation.

4. Evidence is advanced to support the suggestion that the decrease in plasma components is related to the extra drain of colostrum synthesis rather than to any hormonal changes associated with parturition.

We are indebted to the following: the Department of Scientific and Industrial Research for financial assistance towards the cost of this investigation; Mr R. M. Patchell and the farm staff of The Dairy Research Institute for the collection of milk samples; Miss Fay Frecklington for help with much of the analytical work; one of us (S.Y.T.) to the Royal Society, the Nuffield Foundation, and the Board of The Dairy Research Institute (N.Z.) for a travelling fellowship during the tenure of which the work reported here was carried out.

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660. PROPERTIES AND COMPOSITION OF COLOSTRUM FROM EGYPTIAN BUFFALOES AND COWS

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(With 2 Figures)

The role of colostrum in sustaining the health and well-being of the newborn calf has received increased attention in recent years. A general picture of its gross composition and a recognition of its variability emerged from early studies such as that by Overman & Sanman (1). More recently, several studies on the changes of the principal constituents of cows' colostrum have been reported by Garret & Overman (2), Parrish, Wise, Atkeson & Hughes (3), Parrish, Wise, Hughes & Atkeson (4) and Vanlandingham, Weakley & Ackerman (5). The present investigation was initiated because of the lack of information concerning the composition and properties of buffaloes' colostrum.

EXPERIMENTAL

The experimental animals

Samples of colostrum were obtained from each of six buffaloes immediately following parturition, then regularly at each milking until the tenth milking and then at the fifteenth day. Samples of first colostral secretion were also obtained from six cows. The animals were selected from the herds of the Faculty of Agriculture at the University of Alexandria and were free of gross abnormalities. This investigation was made during the winter season, when the typical ration fed was composed mainly of the green foliage berseem (*Trifolium alexandericum*) in liberal amounts.

Sampling procedures

The samples were obtained immediately after calving, usually within 4 hr. Samples that were not analysed immediately after collection were stored at a temperature not exceeding 4° C. and for a period not exceeding 24 hr. All samples were adjusted to a temperature of 20° C. and were well mixed before analysis. No preservatives were used.

Analytical methods

Each sample was analysed for specific gravity, acidity, pH, and contents of chlorine, total solids, fat, lactose and various nitrogenous components. Briefly, the analytical methods were as follows:

Specific gravity: determined at 20° C., using Westphal's balance.

Acidity: determined by the standard titration method, the results being expressed as percentage lactic acid.

pH: determined electrometrically, using a glass-electrode pH-meter.

Chlorine: determined by the Sharp and Strubble method as recommended by the Milk Industry Foundation's 'Laboratory Manual' (6).

Total solids: approximately 2 g. of the sample were weighed into a flat-bottomed dish containing fine dry sand and heated at a temperature of 100° C., until constant weight was reached.

Fat: determined by the Gerber method.

Lactose: determined by the gravimetric copper reduction method as recommended by the Association of Official Agricultural Chemists (7).

Nitrogenous components: total N, casein N, albumin N, globulin N, proteose-peptone N and non-protein N were determined by Rowland's (8) semi-micro Kjeldahl procedures.

RESULTS AND DISCUSSION

Table 1 summarizes the results obtained for first colostral secretions from six buffaloes and six cows.

In all the properties and constituents studied, both buffaloes' and cows' colostrum varied considerably in composition. Reports in the literature by Overman & Sanman (1), Shah (9), Parrish *et al.* (3, 4), Garret & Overman (2), Vanlandingham *et al.* (5) and Rowland, Roy, Sears & Thompson (10) also point to variation as a general characteristic of cows' colostrum. The results obtained by Rowland *et al.* (10) for cows' colostrum are similar to those obtained for most of the constituents in the present work.

Table 1. *Properties and composition of first colostral secretion from Egyptian buffaloes and cows*

	Buffaloes			Cows		
	Max.	Min.	Average	Max.	Min.	Average
Specific gravity	1·0728	1·0632	1·0685	1·0670	1·0559	1·0624
Acidity (%)	0·410	0·340	0·380	0·460	0·310	0·400
pH	6·30	6·00	6·15	6·40	6·25	6·35
Chlorine (%)	0·183	0·138	0·161	0·189	0·115	0·150
Total solids (%)	29·13	23·94	26·99	25·76	18·62	22·67
Fat (%)	6·40	3·96	5·22	6·45	5·34	5·91
Lactose (%)	4·11	2·78	3·39	2·57	1·68	1·59
Total N (%)	2·892	2·372	2·721	2·640	1·532	2·181
Casein N (%)	0·872	0·691	0·781	0·769	0·461	0·671
Albumin N (%)	0·198	0·126	0·154	0·198	0·119	0·150
Globulin N (%)	1·764	1·287	1·497	1·535	0·822	1·198
Proteose-peptone N (%)	0·348	0·113	0·231	0·075	0·028	0·044
Non-protein N (%)	0·066	0·049	0·058	0·088	0·063	0·079

Buffaloes' colostrum compared with cows' colostrum

The differences between buffaloes' and cows' colostrum in specific gravity, acidity, pH and chlorine were very small. The two types of colostrum had approximately the same levels and the same range of variations in most of these properties. Buffaloes' colostrum had a higher level of total solids than the cows' colostrum, and it was mainly the lactose, globulin N and proteose-protein N that contributed to that difference (Table 1).

The buffaloes' colostrum had a wider range of fat content than the cows' colostrum. The average fat content of 5·22% found here for buffaloes' colostrum is below the average of 6·50% for normal buffaloes' milk. This is contrary to the corresponding results for cows' colostrum where an average of 5·91% fat was obtained, which is much higher than the average of 3·5% in normal cows' milk. This may be considered one of the main differences between the two colostrums.

The buffaloes' colostrum was much richer in lactose than the cows' colostrum.

The total nitrogen content of the buffaloes' colostrum was higher than that of the cows' colostrum. The difference was due mainly to higher contents of globulin N and proteose-peptone N in the buffaloes' colostrum. The two colostrums had approximately the same level of albumin N, but casein N was slightly higher in the buffaloes' colostrum.

Changes in the properties and composition of buffaloes' mammary secretion during the transition period

The results presented in Table 2 show the changes that occurred in some of the properties and constituents of buffaloes' mammary secretion during the transition period from colostrum to milk. They are averages of the results obtained with six buffaloes.

Table 2. *Changes in properties and composition of buffaloes' mammary secretion during the transition period from colostrum to milk*

	Number of milking							
	1st	2nd	3rd	4th	5th and 6th	7th and 8th	9th and 10th	15th day
Specific gravity	1·0685	1·0410	1·0351	1·0338	1·0330	1·0320	1·0335	1·0323
Acidity (%)	0·380	0·370	0·230	0·250	0·210	0·130	0·110	0·125
pH	6·15	6·20	6·50	6·70	6·60	6·85	6·80	6·70
Chlorine (%)	0·161	0·130	0·119	0·104	0·093	0·073	0·068	0·082
Total solids (%)	26·99	24·89	16·75	17·03	16·71	15·44	16·15	16·61
Fat (%)	5·22	4·84	5·92	6·01	6·03	6·22	6·85	6·81
Lactose (%)	3·39	4·32	4·11	4·59	4·52	4·73	4·65	4·86
Total N (%)	2·721	2·171	1·084	0·984	0·876	0·602	0·676	0·624
Casein N (%)	0·781	0·592	0·538	0·553	0·558	0·456	0·484	0·476
Albumin N (%)	0·154	0·178	0·123	0·127	0·075	0·022	0·059	0·030
Globulin N (%)	1·497	1·269	0·347	0·215	0·169	0·028	0·031	0·026
Proteose-peptone N (%)	0·231	0·087	0·032	0·046	0·037	0·044	0·061	0·052
Non-protein N (%)	0·058	0·048	0·044	0·043	0·037	0·039	0·041	0·033

Specific gravity. The specific gravity decreased rapidly, with most of the change being completed before the fourth milking. This is comparable to the results obtained by Parrish *et al.* (4) with cows' colostrum.

Acidity. The results in Table 2 show a slow and gradual decrease in acidity until a normal level at the seventh and eighth milkings was reached. This is in accordance with the results obtained by Engel & Schlag (11) for cows' colostrum.

pH. The pH followed an upward trend, with the lowest pH value at the first milking and the highest at the seventh and eighth milkings. McIntyre, Parrish & Fountaine (12) reported that the pH of cows' mammary secretions increased from pH 6·28 to 6·50 over the first few days following parturition.

Chlorine. A gradual decrease in the chlorine content occurred from a high level of 0·161% at the first milking to approximately the normal level at the fifth milking.

Total solids. The total solids content was highest at the first and second milkings, but dropped to approximately the normal level in buffaloes' milk at the third milking. This sharp decrease at the third milking was due mainly to a decrease in total nitrogen content. This is not in accordance with the results obtained for cows' colostrum by Parrish *et al.* (4), who observed that the sharpest decrease in the total solids content usually occurred at the second milking.

Fat. The changes in fat content were relatively slight, but it tended to be somewhat lower during the first two than in the later milkings. This is not in agreement with the findings reported for cows' colostrum by Parrish *et al.* (4), who observed that fat content was the most variable constituent of the mammary secretions during the transition period, with a general tendency to be higher at the first two milkings.

Lactose. The results in Table 2 indicate that the lactose content at the first milking was low and that changes from the second milking onwards were relatively slight. This is not in agreement with the findings reported for cows' colostrum by Parrish *et al.* (4), who found a gradual increase in the lactose content from a very low level at the first milking to higher levels at the following milkings.

The nitrogenous components. The order of changes in the nitrogenous components of buffaloes' mammary secretions during the transition period are shown in Figs. 1 and 2.

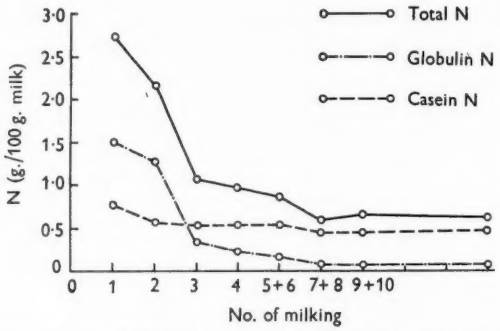

Fig. 1. Changes in the contents of total N, casein N and globulin N in buffaloes' mammary secretion during the transition period from colostrum to milk.

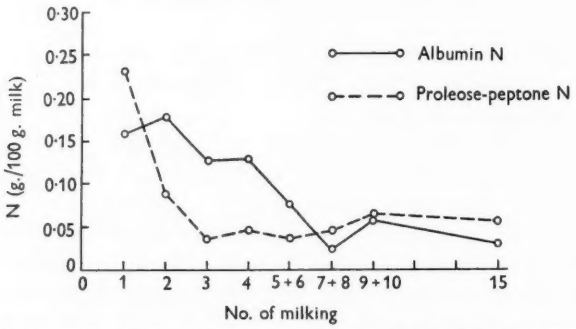

Fig. 2. Changes in the contents of albumin N and proteose-peptone N in buffaloes' mammary secretion during the transition period from colostrum to milk.

Total N, globulin N and proteose-peptone N all dropped sharply from relatively high levels at the first two milkings to low levels at the third milking. Albumin N changed more slowly until the fourth milking. The casein N showed a decrease at the second milking, but changed relatively slightly thereafter. The non-protein N tended to decline during the transition period. The changes in the total N were caused mainly by changes in the globulin N, a fact that can be observed from the curves in Fig. 1.

Parrish *et al.* (3) found that the contents of total N, casein N and albumin plus globulin N in cows' mammary secretions decreased during the change to normal milk. Their results agreed with previous reports by Growther & Raistrick (13) and Sivasubramanian & Dover (14). Parrish *et al.* (3) observed that the main decline occurred by the fourth to the sixth milkings.

SUMMARY AND CONCLUSIONS

Buffaloes' colostrum compared with cows' colostrum

The properties and composition of buffaloes' colostrum have been compared with those of cows' colostrum. The most significant differences found between the two may be summarized as follows:

(1) Buffaloes' colostrum was richer in total solids, lactose, total N, globulin N and proteose-peptone N than cows' colostrum.

(2) Buffaloes' colostrum had a much wider range of fat content than cows' colostrum. A distinct difference between the two colostrums lay in the fact that while buffaloes' colostrum was definitely poorer in fat content than normal buffaloes' milk, cows' colostrum was richer in fat content than is normal cows' milk.

Changes in buffaloes' mammary secretion during the transition period from colostrum to milk

The following is a summary of the most significant trends in the properties and composition of buffaloes' mammary secretion during the transition period:

(1) The specific gravity, acidity and chlorine decreased until they reached a normal value at the fifth milking. This compares well with the trends reported in the literature for cows' mammary secretion during the transition period.

(2) The pH increased gradually until it reached its normal level before the fifth milking.

(3) The highest level of total solids occurred at the first and second milking, and dropped sharply at the third milking to the level normally found in buffaloes' milk.

(4) Changes in the lactose content from the second milking onwards were very slight. This contrasts with the known trend during the transition period in cows, when a gradual increase from a low level at the first milking to higher levels at the following milkings is noticeable.

(5) The total N, globulin N, albumin N and proteose-peptone N decreased from relatively high levels at the first and second milkings to lower levels at later milkings, and thus closely followed the trend shown by the total solids.

Buffaloes' mammary secretion may be considered comparable in its composition to normal buffaloes' milk and suitable for human consumption from 5 to 7 days after parturition.

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REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION F. MILK-BORNE DISEASE

GENERAL

The problem of milk-borne disease is so closely linked to infections amongst domestic animals that a brief account of certain review papers and new books on animal diseases is essential. Jezeski (1) has discussed particular aspects of the microbiology of dairy products, and one section of this paper deals with recent work which has been done on pasteurization and the thermal destruction of certain pathogens such as *Coxiella burnetti*, *Brucella abortus* and *Escherichia coli*. He has emphasized the difficulties which prevent clear-cut conclusions on the problem, and has suggested that naturally infected milk should be employed in the experiments rather than milk which has been contaminated with bacteria and viruses which have been cultured in the laboratory. Southwood (2), in a conspectus of some clinical and preventive aspects of zoonoses in Australia, has noted that many of the vague illnesses and fevers occurring in human beings may have as their original source an animal host. Brucellosis, leptospirosis, rickettsial and virus diseases, in particular, are often transmitted to man from domestic animals. In a review of disease in dairy cattle, Blackburn (3) has discussed mastitis and contagious abortion in great detail. So far as mastitis is concerned *Streptococcus agalactiae* has little association with disease in human beings, but in quite a number of outbreaks of staphylococcal food poisoning the original source must be the cow's udder. In the section of the paper on contagious abortion he has discussed very fully the diagnosis and bacteriology of the disease, and the methods employed in vaccinating cows against it. Chalmers (4), in the fourth edition of his book on bacteria in relation to the milk supply, has dealt with the pathogenic organisms transmissible to man through milk and dairy products under (*a*) organisms originating in the cow, and (*b*) those originating from persons handling the milk. He has called attention to the fact that the Milk and Dairies Regulations 1949 to 1953 and the Milk and Dairies (Scotland) Act, 1914, prohibit the sale of or use of milk known to be infected or suspected of being infected with the organisms of a notifiable or bovine disease until it has been treated in a manner satisfactory to the Medical Officer of Health. Furthermore, the *Brucellosis melitensis* Order 1940 has empowered the authorities to slaughter animals infected with *Br. melitensis*. Davies (5) gave an account of the annual scientific conference organized by the East African Medical Research Scientific Advisory Committee held at Kampala, Uganda. The conference was concerned with the inter-relationship of disease in animals and man, and the symposia on bacterial infections, protozoal infections, and virus diseases showed how intricate these relationships can be.

The reports of the Chief Medical Officer of the Ministry of Health (6, 7) and the Public Health Laboratory Service (8, 9) summarize the occurrences of food-poisoning outbreaks in England and Wales associated with all types of food vehicles. From these, the particulars dealing with food poisoning in which milk and milk products have been incriminated have been abstracted and are given in Table 1. For the years 1953 and 1954 twenty outbreaks were associated with fresh, dried and canned milk. In nine of these staphylococci were the causative agents, and in six, *S. typhi murium*. There were four outbreaks due to cheese and all were caused by staphylococci. As regards food substances in which milk or cream was used to some extent, in trifles, ice cream, cream-filled cakes, and custards, twenty out of forty-one outbreaks were due to salmonellae and eight to staphylococci, and in twelve the organismal cause was not identified. It is probable that in many of the *Salmonella* infections the organisms were not derived from the milk originally but were introduced by contamination at some stage in the preparation of the sweetmeat. *Clostridium welchii*, which has been established by Hobbs, Smith, Oakley, Warrack & Cruickshank (10) as a frequent cause of food poisoning associated with prepared meats, is not recorded as a cause of

food poisoning associated with milk and milk products. In the U.S.A., however, *Cl. botulinum* has been proved to be an occasional cause of food poisoning linked with cheese, particularly of the 'cottage' type. Thus many years ago Nevin (11) reported a food-poisoning incident in which three individuals ate cottage cheese and died. Again Meyer & Eddie (12) described 483 outbreaks of botulism. In five incidents cheese was the affected food, and of eighteen persons involved eight died. The authors stated that cottage, Limburger, and Neufchatel cheese prepared in the home are all suitable for the development of botulinus toxin within a few days of their

Table 1. *Summarized reports of Chief Medical Officer and the Public Health Laboratory Service for England and Wales on food poisoning*

		S. typhi murium	Other Salmonella	Staphylococci	*Cl. welchii*	Other organisms	Not recorded	All agents
Milk: fresh, dried and canned	1953	5	—	4	—	—	3	12
	1954	1	—	5	—	—	2	8
Cheese	1953	—	—	1	—	—	—	1
	1954	—	—	3	—	—	—	3
Sweetmeats, trifles, ice cream, custard, cream buns	1953	13	2	3	—	—	9	27
	1954	4	1	5	—	1	3	14

preparation. More recently, Meyer & Eddie (13) described a fatal case of botulism in a 53-year-old man who ate two and a half ounces of Liederkranz cheese containing the type B organism. It was estimated that 1 g. of the infected cheese contained 50 M.L.D. of toxin and that the patient ingested 3500 M.L.D. of toxin. The U.S.A. reports on food poisoning for the years 1953 and 1954 have been published by Dauer & Sylvester (14,15), and the appropriate abstract is given in Table 2. These reports, in comparison with the British ones, show relatively fewer outbreaks, and this is particularly so when the total populations are taken into consideration.

Table 2. *Outbreaks of disease in the U.S.A. due to milk and milk products*

Year	No. of outbreaks	No. of cases	Dairy product transmitting the infection	Infectious agent
1953	4	97	Ice cream	1 outbreak *S. typhi murium*
			Ice cream	1 outbreak *S. montevideo*
			Cream cheese	1 outbreak dysentery organisms
			Home-made cheese	1 outbreak staphylococci
1954	9	200	Egg-nog	1 outbreak *S. typhi murium*
			Ice cream	1 outbreak staphylococci
			Cream cheese	1 outbreak not established

The progressive increase in the number of food-poisoning incidents in this country has led to the introduction of a new Act (Food and Drugs Act, 1955) which became operative in England and Wales on 1 January 1956, and a similar one for Scotland on 1 August 1956. Part II of the Act defines synthetic creams. Where the material contains milk products it will be known as 'reconstituted cream' and when it contains no milk products 'imitation cream'. The introduction of synthetic cream during the early years of the last war was responsible for many outbreaks of disease, particularly paratyphoid fever and food poisoning due to staphylococci and salmonellae. Hobbs & Smith (16), in a series of experiments designed to test the capacity of such products to support the growth of various pathogenic organisms, found that protein was an essential ingredient for this purpose. They suggested that growth in synthetic cream might be inhibited by: (*a*) limiting the ingredients to cooking fat or other fatty material, excluding margarine or butter, an emulsifying agent such as methyl cellulose, sugar and salt; and (*b*) by the addition to the

synthetic cream of a bacteriostatic agent such as hydrogen peroxide. The concentration of the H_2O_2 necessary to inhibit the growth of organisms was found to be dependent on the ingredients. When butter, milk and egg yolk were present three times the concentration of H_2O_2 was necessary as when emulsified fat without protein was used. The inhibiting power of 0·012% H_2O_2 remained effective in a fat emulsion cream and also in a laboratory prepared cream containing margarine, egg and milk, for 3 weeks at 4° and 20° C. At higher temperatures the stability of the oxidizing agent was doubtful.

TUBERCULOSIS

There has been published a Ciba Foundation Symposium (17) on experimental tuberculosis with an addendum on leprosy. The volume contains numerous papers by many well-known authorities and the appropriate summaries of the discussions on them. These papers deal with the chemistry of the tubercle bacillus, tissue reactions, serology, immunology, and pathogenicity. The book will be a most valuable source of reference to all interested in the many laboratory aspects of tuberculosis. The Joint Tuberculosis Council (18) has issued a report on the changing character of tuberculosis. The report emphasized the fact that this country is not alone in having experienced a declining mortality without a corresponding decrease in notifications. The divergence of the notifications and mortality rates began in this country before satisfactory chemotherapy was established, but this form of treatment has now probably accentuated the divergence. The elimination of tuberculosis due to infected milk has been a helpful factor, and the report stated that Baar, who carried out a series of post-mortems on children dying of tuberculosis in the Birmingham Children's Hospital, found primary disease, presumably of bovine origin, in the abdomen in eight cases in 1932–40, in twelve in 1941–43, in thirteen in 1944–46, in seven in 1947–49, and none in 1950–54. The report stated also that the clinical and mortality data agree that bovine tuberculosis is declining in many areas in this and other countries with consequent reduction in the incidence of abdominal and glandular tuberculosis and to a lesser extent tuberculous meningitis. The Chief Medical Officer for England and Wales stated, in his report for the year 1954 (19), that about 87% of the milk retailed was subject to heat treatment compared with 69% in 1945–46. The maximum incidence of all types of tuberculosis occurred in 1949, and since that time there has been a marked decrease and this has occurred despite the extensive use of mass X-ray diagnostic methods. Table 3, which has been adapted from those issued in the Chief Medical Officer's reports, shows something of this satisfactory trend.

Table 3. *Tuberculosis in England and Wales*

Year	Total notifications, all forms	Deaths: Respiratory M.	Respiratory F.	Respiratory All	Other forms M.	Other forms F.	Other forms All	All forms M.	All forms F.	All forms All
1953	44,000	5447	2466	7913	517	472	989	5964	2938	8902
1954	40,000	4944	2125	7069	448	380	828	5392	2505	7897
Percentage decline of deaths in 1954:										
From 1939		61	76	67	80	79	80	64	77	69
From 1948		57	72	63	70	73	71	59	72	64

The figures indicate that the actual incidence of the disease has declined to a lesser extent than the mortality rate. Chalmers (20) has published a review of the milk supply in Scotland. By September 1954, 87·3% of registered producers in Scotland held licences for 'Certified' or 'Tuberculin Tested' milk. In addition, 81 million gallons of milk were produced during the year from non-licensed herds which were attested, thus 98% of the milk was obtained from cows which had passed the tuberculin test. During this period 1950 to 1953, 1171 samples of 'Certified' and 'Tuberculin Tested' milk were examined for tubercle bacilli by guinea-pig inoculation. Of these

only four were found to be positive. On the other hand, 5·8% of samples of 'Standard' milk (now withdrawn) and 2·3% of samples of non-designated milk showed the presence of this organism. Detailed complementary papers on the control of human and bovine tuberculosis in Denmark have outlined the steps taken and progress of the projects. Groth-Petersen(21) has described the control of the disease in humans, and has mentioned that since 1946 when tuberculosis in cattle was practically eliminated the population has been offered B.C.G. vaccination and, by 1953, 60% of those aged 15–29 years, and one-third of the whole population had received the vaccine. Plum(22) has described the control of bovine tuberculosis and recalled the introduction by Barry in 1891 of the subcutaneous tuberculin test. Later, in 1898, a law was passed enabling the authorities to slaughter animals proved to be suffering from tuberculous mastitis. The intradermal test was introduced by Christiansen in 1910, but difficulties in the interpretation of reactions continued for many years. An eradication scheme under the control of the Veterinary Department was eventually introduced in 1932 on a voluntary basis, and in 1942 further legislation was introduced in order to complete the eradication. In 1953, 99·9% of all herds were free from tuberculosis and only twenty-four re-infections were recorded. Groth-Petersen, Viskum & Wilbek(23) have studied the size of the tuberculin reactions among populations where cattle tuberculosis occurred and in areas where bovine disease was absent. In the areas where bovine tuberculosis had recently occurred the tuberculin reactions in human beings were definitely larger and more marked than in individuals living in areas from which the bovine disease had been absent for a long period. Lethem(24) has made an interesting analysis of the statistics

Table 4. *Abdominal tuberculosis in children under five years of age*

	1921		1953	
	Rates per million	Number of deaths	Rates per million	Number of deaths
London	136	51	4	1
County boroughs	431	490	1	1
Urban districts	336	390	1	2
Rural districts	252	176	12	8
		1107		12

(Table 4) in connexion with the deaths from abdominal tuberculosis in England and Wales. Most cases of this type of infection are due to the bovine type of bacillus, and the very marked decrease in the number of deaths clearly illustrates the value of the measures which have been taken to eradicate the disease in bovines. In 1921 the deaths, from abdominal tuberculosis, of children under five years of age totalled 1107, whereas in 1953 there were only twelve. There was still a higher incidence in rural areas, but the disease has now been reduced to negligible proportions.

SALMONELLA INFECTIONS

(1) *Enteric fevers*

The occurrence of large outbreaks of typhoid and paratyphoid fever as a result of milk infection is now relatively infrequent. In 1940 the use of natural cream was prohibited in Great Britain and synthetic cream substitutes were introduced. In the Annual Report of the Chief Medical Officer of the Ministry of Health for 1950(25) it was stated that nearly every outbreak of paratyphoid which had occurred since October 1940 was due to infected synthetic substitutes. Outbreaks of food poisoning due to this material contaminated with various types of salmonellae were also frequent. A report in the Bulletin of W.H.O.(26) gave a statistical analysis of the occurrence of typhoid and paratyphoid fevers in many countries from 1950 to 1954, the data being collected by the epidemiological and statistical service of the organization. In general, there was a downward trend of both typhoid and paratyphoid infections in many countries and

in some there was now a pre-eminence of paratyphoid over typhoid fever. Conclusions were difficult to elicit, but it would appear that in temperate countries where there was a large urban population supplied with efficient water supplies, the prevalence of water-borne typhoid was very low, whereas food-borne paratyphoid, though decreasing, had not reached a negligible level. Typhoid fever remained a more serious problem in predominantly rural areas in both temperate and tropical countries. The spoilage of many cans containing imported sterilized cream was reported by Sandford (27). *Salmonella typhi* (Vi-phage type E1) was isolated from one can, and further investigations showed that 17% of 1955 cans of the same consignment contained numerous sporing and non-sporing bacteria, but only one suggestive case of typhoid was reported as being due to eating this material. This fortunate result was probably due to the fact that the cream in the contaminated cans had such a bad smell and taste that the contents were discarded. According to the Annual Report of the Chief Medical Officer for 1954 (28) inquiries showed that the lids on the cans had been imperfectly closed. Further, at the time this particular batch was processed, the usual supply of pure water was not available and impure water from a shallow well, subject to pollution, had been used for cooling purposes. An epidemic of paratyphoid in Lancaster, Pa., U.S.A., was described in an editorial article (29). Some 200 persons had clinical evidence of the disease, and between forty and fifty more had inapparent infections. As the infected milk was distributed to many of the local schools a large number of young people were involved, though the disease, in most cases, was of a very mild nature. The report does not state how the milk was infected, but as soon as the dairy was incriminated the supply was stopped. The occurrence emphasized the efficiency of the full-time public health staff in investigating the outbreak and suggested that a dairy controlled by the Department of Agriculture with little reference to the local public health officials was not a satisfactory arrangement.

(2) Salmonella *food poisoning*

The prevalence of *Salmonella* infections, particularly in bovines, must be a deciding factor governing the occurrence of outbreaks due to milk. Cayton (30), in a paper on animal sources of gastro-intestinal infections, gave figures showing the relative frequency of Salmonellae in the faeces of domestic animals (Table 5) and another (Table 6) showing the relative importance of different species of domestic animals as reservoirs of infection. According to these figures,

Table 5. *Frequency with which Salmonellae were isolated from faeces of adult healthy domestic animals*

Species of animal	Number examined	Number positive for *Salmonella*	Percentage positive
Turkey	650	16	2·50
Goose	100	2	2·00
Duck	500	6	1·20
Pig	600	4	0·67
Chicken	750	5	0·67
Cow	750	3	0·40
Horse	500	1	0·20
Sheep	500	0	0·00

Salmonellae were only obtained from 0·40% of specimens of faeces from cows, and with a total population of almost 7,800,000 animals in England and Wales it was calculated that only some 31,000 could be carriers. Lennox, Harvey & Thomson (31) described a milk-borne outbreak of infection due to *S. typhi murium* in a school with 250 pupils, all under the age of 10 years. Sixty-four of the pupils had definite symptoms and all sickened between 5 and 6 February 1954. The common article of food was school milk, and the school was the only one to receive its supply from a farm which supplied, in addition, three households. The T.T. unpasteurized milk was bottled at the farm. The farmer, his wife, and two children were found to be excreting *S. typhi murium* in their faeces, though none gave a history of illness. In addition to those showing symptoms of

infection twenty-four children showing no symptoms excreted *S. typhi murium* in the faeces. Repeated examination of those positive showed the rate of clearance from infection was slow for 3 weeks and then progressed more rapidly. Galton, Smith, McElrath & Hardy (32) obtained faecal cultures from cattle on seven dairy farms in Florida. The 596 cultures taken from five herds all gave negative results. There were two positive (*S. give* and *S. bareilley*) in one herd of fifty cows and in another large herd of 550 animals there were fourteen positive (two *S. give*, two *S. anatum* and ten *S. kentucky*). In one group of twenty-six calves in this latter herd eight were positive.

Table 6. *The relative importance of different species of animals as reservoirs of* Salmonella *infections*

Species of animal	Population in England and Wales, September 1953	Number positive	Percentage positive
Turkey	1,239,000	30,975	2·50
Goose	677,000	13,540	2·00
Duck	1,602,000	19,464	1·20
Pig	4,458,000	29,868	0·67
Chicken	52,405,000	351,776	0·67
Cow	7,856,000	31,424	0·40
Horse	500,000	1,000	0·20

A new type of *Salmonella* associated with acute enteritis and sometimes transmitted through the agency of milk has been described by Gray & Harvey (33), Grant & Norton (34) and Macdonald, Price & Taylor (35). The organism has the antigenic structure IV, V, XII:b- and is thus very similar to *S. paratyphi* B. but it does not ferment dulcite and it is monophasic. Gray & Harvey described an occurrence in a children's nursery in which two children had enteritis. Further investigation showed the same organism was present in the faeces of fifteen out of twenty-two children, and three out of sixteen adults. The exact method of infection was not determined, but the farmer supplying the milk had a brief attack of enteritis 4 days before the children fell ill, and the same organism was obtained from his faeces. The outbreak described by Grant & Norton was not associated with milk, and the paper by Macdonald *et al.* gave a description of the occurrence of this type of infection in England and Wales generally, and a discussion on the biochemical and serological characteristics of the organism.

INFANTILE DIARRHOEA DUE TO SPECIFIC TYPES OF *ESCHERICHIA COLI*

The association of specific types of *E. coli* with infantile diarrhoea continues to be the subject of extensive investigation. Furthermore, a great deal of work has been done to correlate the types found in man with those found in animals. Lovell (36) has reviewed intestinal disease in young calves with particular reference to infection with *E. coli*, and the epidemiology of this disease has many points in common with the disease as it affects infants. *E. coli* types O 26, O 55, O 86, O 114, and O 119 have all been described as the cause of outbreaks of diarrhoea in infants and all have been isolated from cattle. In addition O 25, O 44, O 111, O 125, O 126, O 127 and O 128 have also been isolated from cases of infantile diarrhoea, but so far their occurrence in animals has not been reported. The position as regards human infections has been discussed by Smith (37). It would seem that on occasion the vehicle of infection might be milk. Small outbreaks of disease and sporadic cases occurring in rural and semi-rural areas are often due to the O 26 type, and as this organism has often been isolated from calves with 'white scours', milk-borne infection may be a possibility. Thomson (38) obtained 1316 samples of milk from supplies delivered to a pasteurizing plant and twelve or approximately 1% showed types of *E. coli* associated with infantile gastro-enteritis. Four strains of O 55:H 21, four of O 26:H 11, one of O 128:H 12, two of O 55:H? and one of O 119:H 27 were obtained. The author regards unheated cows' milk as a dangerous food for babies, and believes that summer diarrhoea was probably for the most part milk-borne.

STAPHYLOCOCCAL FOOD POISONING

Staphylococcal food poisoning is being reported with increasing frequency, and the original source of infection can be either the cow or some person handling the milk or milk preparation. Price *et al.*(39) have undertaken a study of the value of phage typing, antibiotic sensitivity, and haemolysis on blood agar plates, of strains of *Staphylococcus aureus* isolated from bovine mastitis in order to determine the value of these tests in distinguishing between new infections and relapses. Organisms of two phage types were rarely found in one quarter of the udder, even though three or four distinguishable types were present in the herd. In quarters infected for many months phage type and penicillin sensitivity were found to be very stable. Parker(40), in a paper on staphylococcal food poisoning occurring in the Manchester area between January 1949 and December 1952, described twenty bacteriologically proved outbreaks, but only in three instances were they associated with milk or milk products. The suspected foods were custard, sour cream and cream cheese, and Cheshire cheese, and fifteen, twenty-four and nineteen individuals were affected respectively. In the Annual Report of the Chief Medical Officer for England and Wales for 1953(41) a description is given of large outbreaks of staphylococcal food poisoning conveyed through the agency of spray-dried skim milk. There were four outbreaks associated with schools in Surrey, three with schools in London, and one with a school in Yorkshire. In all, 1190 individuals showed symptoms of illness, though many more were exposed to the possibility of infection. Investigation showed that the skim milk had been supplied by one manufacturer, and from various samples *Staph. aureus* phage type 42E/53W was isolated. These outbreaks were the subject of further investigation by Anderson & Stone(42), who examined the conditions at the plant manufacturing the dried milk. They showed that the dried powdered milk had a high bacterial content, staphylococci of phage type 42E/53W were found in it. They believed that the enterotoxin was formed by the staphylococci in the balance tank where the milk was often kept warm for several hours prior to being passed into the drying chamber. An annotation in the *Lancet*(43) commented on the outbreaks and on the work carried out by Anderson & Stone. Attention was called to the fact that staphylococci are at least as common on the bovine teat as they are on human hands and equally as difficult to remove. Furthermore, there was the possibility that owing to the widespread use of penicillin in the treatment of streptococcal mastitis, staphylococci may become more common than they were. Steede & Smith(44) gave an account of two small related outbreaks of food poisoning in which the infected agent, clotted cream, was prepared from the milk of a cow found to be excreting *Staph. pyogenes* in her milk. In the first outbreak four persons were involved and in the second seven in one family. Clinical examination of the single-house cow failed to show any evidence of a mastitis, but the organism could be readily isolated from a composite sample from the four quarters of the udder. A similar organism was also isolated from the left nostril of the man who milked the cow. Smith(45) had previously shown that most staphylococci isolated from milk belonged to phage type 42D, but the organism isolated from the milker's nostril and from the milk belonged to phage type 53. As this type is frequently associated with human infections, it was believed that the milker had transmitted the organism to the cow's udder. Bravo(46) described an outbreak of staphylococcal food poisoning due to infected ice cream in which eighty-six individuals out of a total of 148 were involved. Within 2–4 hr. of eating the peccant material most individuals affected complained of epigastric pain and repeated vomiting followed later by diarrhoea. In a bacteriological study of Cottage cheese, Lyons & Maltmann(47) found that the survival of *Staph. aureus* was closely linked to the pH. This organism survived for 192 hr. at pH 4·6 in this medium.

BRUCELLOSIS

Since the last review a new book by Loeffler, Moroni, & Frei(48) has been published on 'Brucellosis, a Disease of Man and other Animals'. The monograph consists of almost 200 pages and contains a detailed account of the literature and a classified bibliography of some 800 references. The

methods of transmission, laboratory techniques for diagnosis, the experimental pathology, the epidemiology, and the prophylaxis of the disease are fully described. The most detailed part of the book deals with the manifestations of the disease in human beings, clinical symptoms, differential diagnosis, pathology, and treatment. It is a most comprehensive volume and a desirable reference book. Blackburn (49) has recently reviewed in this *Journal* the subject of contagious abortion. He has dealt with the literature on diagnosis, bacteriology and vaccination, and a further section of the paper describes the latest work on the experimental pathology of the disease. A further paper by Stableforth (50) has described brucellosis in animals and man. It reviews the literature on the signs and symptoms of the disease in bovines and man, it discusses its prevalence, and the routes of transmission. Further sections of the paper deal with the legal powers with regard to infected milk and the control of the disease in bovines by eradication and vaccination. According to Southwood (51) infection of cows with *Brucella abortus* is common throughout Australia, and out of a total population of 170,000 in South Australia it has been estimated that 32,000 are infected. He states that infection of man by *Br. abortus* from milk is probably uncommon but, occasionally, the organism gains entrance through abrasion of the skin of dairymen. According to the *Lancet* (52) the medical officer of health for Gibraltar referred in his report for 1953 to an outbreak of brucellosis. Sixteen cases were notified during the summer—the highest total since undulant fever was made notifiable in Gibraltar in 1905. The disease was known to have been very prevalent there at one time, but no statistics were available prior to 1905, when eleven cases including one death, were notified. In 1906 by-laws were passed enforcing the boiling of milk before sale, and arrangements were made for the quarantining of imported goats and the periodic examination of goats by blood tests. These measures were immediately successful, and though there have been a few small outbreaks since then, the disease is now almost entirely limited to occasional sporadic cases. Officials of the Health Department inquired carefully into the origins of the disease and found clear indications of multiple sources of infection and the local milk supplies were exonerated. Three patients had consumed fresh unboiled goats' milk and fresh cheese when on a picnic at a nearby resort, and another four had eaten fresh goats' cheese which one of them had bought. The source of infection in some of the other patients was undetermined, but they had all consumed milk or fresh cheese at places in the vicinity and were presumably infected from sources outside Gibraltar. Hendricks (53) has given an account of the epidemiology of human brucellosis in Iowa. During the years 1952 and 1953, 720 cases of *Brucella* infection were reported. These consisted of 614 males and 106 females with the majority in the age group 20–39 years. In 61% of cases the causative organism was *Br. suis*, in 26% *Br. abortus*, and in 13% *Br. melitensis*. About 80% of the patients had a history of contact with farm animals, 50% were male farm workers, and 22% were employed in packing houses. Of seventy-four housewives who were affected, 76% were associated with rural areas or farm work. Though *Br. suis* was the main cause of the infections, there was a considerable amount of illness due to organisms from bovine sources. In order to help to eliminate the disease from bovines, vaccination of calves and the tracing of the sources of infections by milk ring tests and serum agglutination tests were extensively carried out. The trends in bovine and human brucellosis in Denmark have been the subject of two papers. Thomsen (54) has described the methods used to try and eradicate the disease from the bovine population. A survey carried out between 1936 and 1944 showed that about 25% of all Danish herds and 11% of the cattle were infected. Later the ring test has made it possible to test the cows in the herds every 3 months, and by vaccination, slaughter, isolation, and disinfection, it has been possible to eliminate infection from herds in a large part of Denmark. Kristensen (55) has described the human aspects of infection with *Br. abortus* and has compared the incidence of the disease in the 5-year period 1928–32 with the incidence in 1951–54. The case rates of human infection dropped from 1·07 to 0·06 per 10,000 in Greater Denmark, from 1·45 to 0·15 in provincial towns, and from 2·98 to 0·63 in rural districts.

Some interesting observations have been made on the bacteriology and serology of organisms belonging to the Brucella group. Thus Braun & Oglesby (56) have called attention to the reports

of naturally occurring Brucella strains which do not conform to the differential characteristics of *Br. suis*, *Br. abortus* and *Br. melitensis*. To investigate these points the mutation rate to dye-resistance was determined for four strains of *Br. suis* and four of *Br. abortus*. Mutants were tested for their virulence in guinea-pigs and the results showed that most dye-resistant mutants could compete successfully with their parent type *in vivo*. A few strains obtained from the Weybridge Laboratories and classified by monospecific sera as *Br. melitensis* had the biochemical reactions of *Br. abortus*—requiring CO_2 and producing H_2S. It was relatively simple to isolate from these strains mutants which did not require CO_2 for their growth. In this country the isolation of *Br. melitensis* from cows in dairy herds has been until recently the signal for slaughter of the herd. So far as can be ascertained, even if cows in a particular area are infected with *Br. melitensis*, there has not been any increase in the incidence of human infections. This is rather remarkable since it is generally held that *Br. melitensis* is a greater source of danger to human beings than *Br. suis* or *Br. abortus*. It would appear that if a policy of slaughter cannot be carried out for *Br. abortus* infection there is little necessity to do it when strains conforming to the melitensis type are isolated. The isolation of *Br. abortus* from milk by direct plating methods has not given satisfactory results due to overgrowth by other organisms. Mair(57) has utilized the antibiotics polymyxin B and penicillin together with acti-dione and gentian violet, incorporated in a horse-blood digest agar base for direct culture. A total of 262 samples from 177 herds were examined by the new cultural method and by guinea-pig inoculation, and 109 were found to be infected. Both methods of testing gave similar results in seventy-two samples, culture gave sixteen positive results when the guinea-pig test was negative, and the guinea-pig test was positive in twenty-one instances in which the cultural method gave negative results. Thus the new medium detected 80·7% of infected samples in 4 or 5 days, whereas guinea-pig inoculation detected 85·3% after 6–7 weeks. Sulitzeanu, Jones & Stableforth(58) have shown that when mice are given a protective dose of an unabsorbed serum prepared against *Br. abortus* or *Br. melitensis* either antiserum will protect them against both organisms. When, however, monospecific sera are used, the animals are only protected against the specific strains. Cruickshank(59) has carried out a series of experiments on the use of the chelating agents, sodium dimethyldithiocarbonate and oxine, in the differentiation of seventy strains of organisms of the brucella group. Filter-paper disks were impregnated with solution of the substances and then placed on liver agar plates which had been inoculated with the various strains. These plates were then incubated at 37° C. in 10% carbon dioxide. Cruickshank concludes: (1) that the pattern of growth and inhibition of the three species as it appears in the carbonate test originally described by Renoux(60) is of some value since *Br. suis* is more resistant than the other two species; (2) and that when oxine is used, strains of *Br. suis* were consistently more resistant than other species.

A working party of the Public Health Laboratory Service(61) has made a report on the value of the milk ring and related tests in the detection of brucella organisms in milk. The related tests included the whey agglutination test, the capillary tube test, and the milk plate test. The conclusions reached were that sole reliance cannot be placed on any milk agglutination test. In 60–75% of samples giving a positive reaction *Br. abortus* could not be demonstrated by guinea-pig inoculation. These tests serve their most useful purpose in the detection of *Brucella* infections in dairy herds and can be used in the laboratory for screening purposes.

LEPTOSPIROSIS

A monograph on the laboratory diagnosis of leptospirosis has been written by Wolff(62). In this volume those interested will find the particulars of the various procedures which can be used for diagnosis of leptospiral infection in man and animals. The techniques described are those which have proved successful already, and the methods employed in overcoming difficulties in culturing and maintaining these rather fastidious organisms are outlined. A section on the present-day classification of the leptospira by serological methods, previously described by Wolff(63) and by

Wolff & Broom (64) is included in the volume. Schlossberger & Brandis (65) have contributed an extensive general review on *Leptospira*. They have discussed morphology, growth requirements, including metabolism and cultivation, and antigenic structure. Weil's Disease and canine leptospirosis are discussed in detail and considerable space is devoted to *Leptospira bovis* and *L. pomona*. A review by McDonald (66) has appeared on leptospirosis in domestic animals with special reference to the disease due to *L. pomona* in cattle, calves and sheep in Australia. There is, however, little additional information available in connexion with the transmission of the disease from animals to man through the agency of milk. From New Zealand, however, come interesting papers on the occurrence of leptospirosis in livestock, and the transmission of the disease to man. In the Wallaceville Area, of 5241 sera from cattle, 534 or 10·27 % were found to give positive agglutination tests. In the Northland Area, Ensor & McClure (67) have noted the occurrence of redwater (leptospirosis) on eighty-two farms, involving 350 calves. In a further paper by Kirschner & Maguire (68) it is shown that leptospirosis is prevalent in man and animals in New Zealand, but, at variance with other parts of the world where rodent reservoirs of infection predominate, domestic farm animals (pigs and cattle) are the main sources. Of 262 patients diagnosed in the laboratory, 200 (80 %) were dairy farmers or members of their families, and the rest had some contact with livestock. It is known that cattle after an acute or subclinical stage of disease may excrete virulent *Leptospira* in the urine for several months. As in brucellosis, the infected urine could serve as a source of human infection through the milk. So far, however, no milk-borne infections have been reported from many countries where bovine leptospirosis is known to be widely spread. The authors found that undiluted milk rapidly killed leptospira. The studies they described in this paper were concerned with the distribution and nature of the antileptospiral principle in milk. They examined 100 samples of cows' milk, twenty of human milk, ten of human colostrum, and ten of goats' milk. They found a destructive factor for virulent and non-virulent mutants of *Leptospira*. It was present in milk from all three species with some variations in titre. The principle is mainly associated with the casein, and in the whey the titre is much lower. The mode of action (rapid dissolution of the organisms) by this factor in milk and its physical properties are similar to, but not identical with, lysozyme. This natural protective agent in milk might explain the absence of milk-borne infections in the many countries where bovine leptospirosis is widely spread.

Q FEVER

Although the exact mode of transmission has not been fully established, epidemiological evidence rather precludes the possibility of the disease being transmitted to any extent through the ingestion of milk or milk products. Infection seems to be contracted through the inhalation of dust contaminated by infected secretions and excretions, and perhaps also by the handling of infected animals. On the other hand, the presence of *Coxiella burneti* has been repeatedly demonstrated in milk by means of guinea-pig inoculation, and milk as an occasional source cannot be excluded. An historical review of the literature on Q fever has been published by Wentworth (69), who gave a detailed account of the etiological and epidemiological studies that have been made. She pointed out that the disease appears to be endemic in the United States, England, Germany, Italy, Turkey, the Balkans, North Africa, Panama and Australia. Infection occurs in domestic animals, as well as in domestic fowls and pigeons, and epidemiological evidence points to the transmission of disease by the inhalation of dust contaminated by infected secretions. Kaplan & Bertagna (70) have contributed a comprehensive article on the geographic distribution of Q fever. The disease has now been found to exist in fifty-one countries in five continents. The most important source of infection was domestic ruminants, and the international traffic in these animals probably accounts for the widespread distribution of sources of infection. The disease has not been recognized in Ireland, the Netherlands, New Zealand, Poland, and the Scandinavian countries, which import few animals. The spread of Q fever from animal to man has been discussed by Stoker & Marmion (71). The authors have reviewed the sources of *C. burneti* in nature, and point out that it has been isolated from ticks, domestic and

wild animals and birds. The transmission from domestic animals to man occurs: (1) by direct occupational contact with parturient sheep, cows or goats; (2) by direct occupational contact with animal products; (3) by residence in proximity to sources of animal infection; (4) by milk; and (5) by concealed exposure. As regards milk it is possible for raw-milk drinkers to be exposed to infection and cases of Q fever should, therefore, occur amongst individuals beyond residential and occupational risks. There is not much evidence that this does happen, and it is suggested that the role of infected milk in the transmission of the disease requires further investigation. Stoker, Brown, Keith, Collins & Marmion (72) and Stoker & Marmion (73) have now isolated *C. burneti* from placentae and wool in an endemic area of Romney Marsh in Kent, and from ticks obtained from the animals in this area. They believe that the endemicity of Q fever in south-east England can be readily accounted for by the consumption of infected cows' milk and contact with parturient sheep or their products. Craddock & Gear (74) have described the occurrence in Kenya of thirteen possible cases of Q fever in which the diagnosis was confirmed in nine instances by serological tests. The disease apparently occurs as a result of occupational contacts rather than the consumption of infected milk. In a working party report of the Public Health Laboratory Service (75) a small outbreak of some fifteen cases of Q fever is described occurring in a rural area of Cardiganshire. Epidemiological investigations excluded case to case infection, infection of milk and water, and visits to a common environment. Serological evidence of infection among sheep was obtained, and it is suggested that these animals were the source. Grist (76), after an extensive series of tests on milk and blood samples, has reported on the incidence of Q fever in the south-west of Scotland in animals, milk, and human beings. Less than 1% of blood samples from sheep and cattle showed any significant antibody titre for *C. burneti*. No less than 471 milk samples (including fifty-seven pasteurized) were inoculated into guinea-pigs. None of the pasteurized samples produced an infection, but seven positive results were obtained from the remaining samples. Serum specimens from 377 cases of adult pneumonia, 123 cases of baby pneumonia, ninety-two cases of virus pneumonia, eighty-four cases of P.U.O. were examined, but only four gave significant antibody titres and only in one instance was an increase in the titre manifest when tests were repeated.

VIRUSES

So far there has been little concrete evidence that virus diseases can be distributed through the agency of milk. With the introduction of new techniques many new viruses have been found in the upper respiratory and intestinal tracts of man. It may be possible in the future to demonstrate some of these in contaminated milk supplies, and the problem of their survival in such a medium is being studied.

Kaplan & Melnick (77) studied the effect of milk and other dairy products on the thermal inactivation of Coxackie viruses. Coxackie viruses from human faeces, and standard mouse-passaged strains, were inactivated in watery suspension by exposure to temperatures of 55° C. for 15 min. and 71·1° C. for 15 sec. Milk, cream, and ice cream increased the thermal stability of the viruses. Strains of human origin suspended in these products were inactivated by HTST pasteurization at 71·1° C. for 15 sec.; holding at 61·7° C. for 30 min. inactivated suspensions in milk and cream but not in ice cream, which last required 15 min. exposure at 65° C. for inactivation. Protection by dairy products was more marked in the case of mouse-passaged strains, which survived both holding and HTST treatments when suspended in cream or ice cream. Texas-1 virus in milk survived exposure to 71·1° C. for 20 sec., but was inactivated within 30 sec. at this temperature. Survival of Texas-1 virus was reduced by dilution of the virus.

These results are discussed in relation to standard methods of heat treatment of dairy products. It is noteworthy that the concentrations of virus used in the experiments were much greater than would be likely through casual contamination in nature. Again Dalldorf (78) has stated that Coxackie and poliomyelitis viruses are relatively resistant to heat and they are even more stable if suspended in dairy products. While standard methods of pasteurization of milk proved effective in destroying several strains of Coxackie viruses, cream, and, to a somewhat lesser degree, ice

cream, provided more protection. High-titred mouse passaged strains of virus were incompletely inactivated if suspended in cream and pasteurized at either 61·7° C. for 30 min. or 71·1° C. for 15 sec.

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